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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: <b>PCT/AU96/00094</b></p> <p>(22) International Filing Date: <b>22 February 1996 (22.02.96)</b></p> <p>(30) Priority Data:</p> <table> <tr> <td>PN 1457</td> <td>2 March 1995 (02.03.95)</td> <td>AU</td> </tr> <tr> <td>PN 6647</td> <td>20 November 1995 (20.11.95)</td> <td>AU</td> </tr> <tr> <td>PN 7274</td> <td>22 December 1995 (22.12.95)</td> <td>AU</td> </tr> </table> <p>(71) Applicant (<i>for all designated States except US</i>): AMRAD OPERATIONS PTY. LTD. [AU/AU]; 17-27 Cotham Road, Kew, VIC 3101 (AU).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (<i>for US only</i>): HAYWARD, Nicholas, Kim [AU/AU]; 13 Prince Street, Paddington, QLD 4064 (AU). WEBER, Gunther [SE/AU]; 17-27 Cotham Road, Kew, VIC 3101 (AU). GRIMMOND, Sean [AU/AU]; 210 Swann Road, Taringa, QLD 4068 (AU). NORDENSKJOLD, Magnus [SE/SE]; Koltrastvagen 19, S-161 37 Stockholm (SE). LARSSON, Catharina [SE/SE]; Dannemoramagatan 10m 3tr, S-113 44 Stockholm (SE).</p> <p>(74) Agents: HUGHES, E., John, L. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).</p>		PN 1457	2 March 1995 (02.03.95)	AU	PN 6647	20 November 1995 (20.11.95)	AU	PN 7274	22 December 1995 (22.12.95)	AU	<p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>	
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(54) Title: A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME

(57) Abstract

The present invention relates generally to an isolated molecule having vascular endothelial growth factor-like properties and to a genetic sequence encoding same. The molecule will be useful in the development of a range of therapeutics and diagnostics useful in the treatment, prophylaxis and/or diagnosis of conditions requiring enhanced or diminished vasculature and/or vascular permeability. The molecule of the present invention is also a useful effector of primary and central neurons and is capable of inducing astroglial proliferation.

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- 1 -

A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE  
ENCODING SAME

- 5 The present invention relates generally to an isolated molecule having vascular endothelial growth factor-like properties and to a genetic sequence encoding same. The molecule will be useful in the development of a range of therapeutics and diagnostics useful in the treatment, prophylaxis and/or diagnosis of conditions requiring enhanced or diminished vasculature and/or vascular permeability. The molecule of the present  
10 invention is also a useful effector of primary and central neurons and is capable of inducing astroglial proliferation.

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOS.) for  
15 the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to  
20 imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Vascular endothelial growth factor (hereinafter referred to as "VEGF"), also known as vasoactive permeability factor, is a secreted, covalently linked homodimeric glycoprotein  
25 that specifically activates endothelial tissues (Senger *et al.*, 1993). A range of functions have been attributed to VEGF such as its involvement in normal angiogenesis including formation of the corpus luteum (Yan *et al.*, 1993) and placental development (Sharkey *et al.*, 1993), regulation of vascular permeability (Senger *et al.*, 1993), inflammatory angiogenesis (Sunderkotter *et al.*, 1994) and autotransplantation (Dissen *et al.*, 1994) and  
30 human diseases such as tumour promoting angiogenesis (Folkman & Shing, 1992), rheumatoid arthritis (Koch *et al.*, 1994) and diabetes related retinopathy (Folkman & Shing, 1992).

- 2 -

VEGF is, therefore, an important molecule making it a potentially valuable target for research into therapeutics, prophylactics and diagnostic agents based on VEGF or its activities. There is also a need to identify homologues or otherwise related molecules for use as an alternative to VEGF or in conjunction with VEGF.

5

In work leading up to the present invention, the inventors sought the multiple endocrine neoplasia type I susceptibility gene (MEN1). Surprisingly, the inventors discovered that a genetic sequence excluded as a candidate for the MEN1 gene was nevertheless a new growth factor having some similarity to VEGF. Furthermore, the growth factor of the 10 present invention is an effector molecule for primary and central neurons.

Accordingly, one aspect of the present invention comprises a biologically isolated proteinaceous molecule comprising a sequence of amino acids which:

- (i) is at least about 15% similar to the amino acid sequence set forth in SEQ ID 15 NO:2; and  
(ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:2.

Another aspect of the present invention provides a biologically isolated proteinaceous molecule having the following characteristics:

- 20 (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:2;  
(ii) exhibits at least one property in common with VEGF.
- 25 A related aspect of the present invention contemplates a biologically isolated proteinaceous molecule having the following characteristics:  
(i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;  
30 (ii) exhibits at least one of the following properties:  
(a) ability to induce proliferation of vascular endothelial cells;  
(b) ability to interact with *flt-1/flk-1* family of receptors;

- 3 -

- (c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

By "biologically isolated" is meant that the molecule has undergone at least one step of  
5 purification from a biological source. Preferably, the molecule is also biologically pure meaning that a composition comprises at least about 20%, more preferably at least about 40%, still more preferably at least about 65%, even still more preferably at least about 80-90% or greater of the molecule as determined by weight, activity or other convenient means, relative to other compounds in the composition. Most preferably, the molecule  
10 is sequencably pure.

Another preferred aspect of the present invention provides the molecule in recombinant form.

- 15 According to this aspect of the present invention, there is provided a recombinant molecule comprising a sequence of amino acids which:
- (i) is at least about 15% similar to the amino acid sequence set forth in SEQ ID NO:2; and
  - (ii) is at least 5% dissimilar to the amino acid sequence set forth in SEQ ID NO:2.

20 A related aspect of the present invention is directed to a recombinant molecule having the following characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with VEGF.

A further related aspect of the present invention contemplates a recombinant molecule having the following characteristics:

- 30 (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;

- 4 -

- (ii) exhibits at least one of the following properties:
- (a) ability to induce proliferation of vascular endothelial cells;
  - (b) ability to interact with *flt-1/flk-1* family of receptors;
  - (c) ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

5

The present invention also contemplates genomic or partial genome clones encoding a proteinaceous molecule having at least about 15% amino acid similarity but at least about 5% dissimilarity to SEQ ID NO:1.

10

The amino acid sequence set forth in SEQ ID NO:2 corresponds to human VEGF (referred to herein as "VEGF<sub>165</sub>"). Accordingly, the molecule of the present invention is VEGF-like or is a homologue of VEGF but comprises an amino acid sequence which is similar but non-identical to the amino sequence of VEGF. Although the present 15 invention is exemplified using a human VEGF-like molecule, this is done with the understanding that the instant invention contemplates the homologous molecule and encoding sequence from other mammals such as livestock animals (e.g. sheep, pigs, horses and cows), companion animals (e.g. dogs and cats) and laboratory test animals (e.g. mice, rats, rabbits and guinea pigs) as well as non-mammals such as birds (e.g. 20 poultry birds), fish and reptiles. In a most preferred embodiment, the VEGF-like molecule is of human origin and encoded by a gene located at chromosome 11q13. The present invention extends, therefore, to the genomic sequence or part thereof encoding the subject VEGF-like molecule.

25 Preferably, the percentage similarity is at least about 30%, more preferably at least about 40%, still more preferably at least about 50%, still even more preferably at least about 60-70%, yet even more preferably at least about 80-95% to all or part of the amino acid sequence set forth in SEQ ID NO:2.

30 In a particularly preferred embodiment, the VEGF-like molecule of the present invention comprises a sequence of amino acids as set forth in SEQ ID NO:4 or is a part, fragment, derivative or analogue thereof. Particularly preferred similarities include about 19-20%,

- 5 -

and 29-30%. Reference herein to derivatives also includes splice variants. Accordingly, the present invention extends to splice variants of SOM175. Examples of splice variants contemplated by the present invention include but are not limited to variants with an amino acid sequence substantially as set forth in at least one of SEQ ID NO:6, SEQ ID

5 NO:8 and/or SEQ ID NO:10 or mutants or derivatives or further splice variants thereof.

Another embodiment provides a recombinant molecule having the following characteristics:

- (i) an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

15 Another embodiment provides a recombinant molecule having the following characteristics:

- (i) an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

Another embodiment provides a recombinant molecule having the following characteristics:

- 25 (i) an amino acid sequence substantially as set forth in SEQ ID NO:8 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one biological property in common with VEGF.

30

Another embodiment provides a recombinant molecule having the following characteristics:

- 6 -

- (i) an amino acid sequence substantially as set forth in SEQ ID NO:10 or having at least about 15% similarity to all or part thereof provided that said amino acid sequence is at least about 5% dissimilar to all or part of the amino acid sequence set forth in SEQ ID NO:2;
- 5 (ii) exhibits at least one biological property in common with VEGF.

Such properties of VEGF include at least one of:

- (a) ability to induce proliferation of vascular endothelial cells;
- (b) an ability to interact with *flt-1/flk-1* family of receptors;
- 10 (c) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.

In accordance with the present invention, a preferred similarity is at least about 40%, more preferably at least about 50% and even more preferably at least about 65%  
15 similarity.

Still a further aspect of the present invention contemplates a peptide fragment corresponding to a portion of the amino acid sequence set forth in SEQ ID NO:4 or a splice variant thereof such as set forth in SEQ ID NO:6, SEQ ID NO:8 or SEQ ID  
20 NO:10 or a chemical equivalent thereof. The biologically isolated or recombinant molecule of the present invention may be naturally glycosylated or may comprise an altered glycosylation pattern depending on the cells from which it is isolated or synthesised. For example, if produced by recombinant means in prokaryotic organisms, the molecule would be non-glycosylated. The molecule may be a full length, naturally  
25 occurring form or may be a truncated or otherwise derivatised form.

Yet another aspect of the present invention is directed to a nucleic acid molecule encoding the VEGF-like molecule herein described. More particularly, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides  
30 substantially as set forth in SEQ ID NO:3 or having at least 15% similarity to all or part thereof or being capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the

nucleic acid sequence having at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence as set forth in SEQ ID NO:3. The nucleotide sequence set forth in SEQ ID NO:3 is also referred to herein as "SOM175". Preferably, the percentage dissimilarity is about 35%, more preferably about 39% and even more preferably about  
5 40-50% or greater.

For the purposes of defining the level of stringency, reference can conveniently be made to Sambrook *et al* (1989) at pages 9.47-9.51 which is herein incorporated by reference where the washing steps disclosed are considered high stringency. A low stringency is  
10 defined herein as being in 4-6X SSC/0.1-0.5% w/v SDS at 37-45°C for 2-3 hours. Depending on the source and concentration of nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 1-4X SSC/0.25-0.5% w/v SDS at ≥ 45°C for 2-3 hours or high stringent conditions considered herein to be 0.1-1X SSC/0.1% w/v  
15 SDS at 60°C for 1-3 hours.

The present invention further contemplates a nucleic acid molecule which encodes a VEGF-like molecule as hereinbefore described having at least 15% nucleotide sequence homology to SEQ ID NO:3. Preferred levels of homology include at least about 40%,  
20 more preferably around 60-70%.

The present invention is further directed to the murine homologue of human VEGF (referred to herein as "mVRF"). The mVRF has approximately 85% identity and 92% conservation of amino acid residues over the entire coding region compared to human  
25 VEGF. The mVRF is encoded by a nucleic acid molecule comprising a nucleotide sequence substantially as set forth in Figure 9.

The VEGF-like molecule of the present invention will be useful in the development of a range of therapeutic and/or diagnostic applications alone or in combination with other  
30 molecules such as VEGF. The present invention extends, therefore, to pharmaceutical compositions comprising the VEGF-like molecule or parts, fragments, derivatives, homologues or analogues thereof together with one or more pharmaceutically acceptable

- 8 -

carriers and/or diluents. Furthermore, the present invention extends to vectors comprising the nucleic acid sequence set forth in SEQ ID NO:3 or having at least about 15%, more preferably about 40% and even more preferably around 60-70% similarity thereto but at least 30% and more preferably around 39% dissimilarity thereto and host 5 cells comprising same. In addition, the present invention extends to ribozymes and antisense molecules based on SEQ ID NO:3 as well as neutralizing antibodies to the VEGF-like molecule. Such molecules may be useful in ameliorating the effects of, for example, over expression of VEGF-like genes leading to angiogenesis or vascularization of tumours.

10

Another aspect of the present invention contemplates a method of inducing astroglial proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- 15 (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astroglial 20 proliferation.

Preferably, the recombinant proteinaceous molecule comprises the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:6.

25 A further aspect of the present invention provides a method of promoting neural survival and/or proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- 30 (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

- 9 -

said administration being for a time and under conditions sufficient to induce astrogial proliferation.

5 Preferably, the recombinant proteinaceous molecule comprises the amino acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:6.

The present invention also contemplates antibodies to the VEGF-like molecule or nucleic acid probes to a gene encoding the VEGF-like molecule which are useful as diagnostic agents.

10

The present invention is further described by reference to the following non-limiting Figures and/or Examples.

In the Figures:

15

**Figure 1** Nucleotide sequence [SEQ ID NO:1] and corresponding amino acid sequence [SEQ ID NO:2] of VEGF<sub>165</sub>.

20 **Figure 2** Nucleotide sequence [SEQ ID NO:3] and corresponding amino acid sequence [SEQ ID NO:4] of SOM175.

**Figure 3** Results of BLAST search with SOM175 protein sequence.

25

**Figure 4** BESTFIT alignment of VEGF cDNA and SOM175 cDNA.

**Figure 5** Multiple alignment of VEGF<sub>165</sub> with SOM175 and its splice variants at the nucleotide level.

30 **Figure 6** Multiple alignment of VEGF<sub>165</sub> with SOM175 and its splice variants at the amino acid level.

**Figure 7** Diagrammatic representation of SOM175 and its splice variants.

- 10 -

**Figure 8(a)** Diagrammatic representation of genomic structure of human SOM175 genomic showing exon/intron map.

**Figure 8(b)** Diagrammatic representation of genomic structure of human SOM175 5 showing exon/intron boundaries.

**Figure 9** Nucleotide and predicted peptide sequences derived from mVRF cDNA clones. Numbering of nucleotides are given on the left, starting from the A of the initiation codon. Amino acids are numbered on the right, starting from the first residue 10 of the predicted mature protein after the putative signal peptide has been removed. The alternately spliced region is double underlined and the resulting peptide sequence from each mRNA is included. A potential polyadenylation signal is indicated in boldface. Start and stop codons of mVRF<sub>167</sub> and mVRF<sub>186</sub> are underlined and a polymorphic AC repeat in the 3' UTR is indicated by a stippled box. The positions of intron/exons 15 boundaries are indicated by arrowheads.

**Figure 10** BESTFIT alignments of human and murine VRF protein isoforms. A: mVRF<sub>167</sub> and hVRF<sub>167</sub>. B: mVRF<sub>186</sub> and hVRF<sub>186</sub> from the point where the sequences diverge from the respective 167 amino acid isoforms. Amino acid identities are marked 20 with vertical bars and conserved amino acids with colons. An arrow marks the predicted signal peptide cleavage site of human and mouse VRF.

**Figure 11** BESTFIT alignment of mVRF<sub>167</sub> and mVEGF<sub>188</sub> (Breier *et al*, 1992) peptide sequences. An arrow marks the signal peptide cleavage site of mVEGF. 25 Identical amino acids are indicated by vertical bars and conservative substitutions by colons. Numbering of amino acids is as described in the legend to Figure 9.

**Figure 12** Comparison of gene structure between VRF (a generic VRF gene is shown since the intron/exon organisation of the mouse and human homologues is almost 30 identical) and other members of the human VEGF/PIGF/PDGF gene family. Exons are represented by boxes. Protein coding regions and untranslated regions are shown by filled and open sections respectively. The hatched region in VRF indicates the

- 11 -

additional 3' UTR sequence formed by alternate splicing of the VRF<sub>186</sub> isoform. Potential alternate splice products of each gene are shown.

5      **Figure 13**    Autoradiogram of a Northern blot of total RNA from various adult mouse tissues (as indicated) hybridised with an mVRF cDNA clone. A major transcript of 1.3 kb was detected in all samples.

10     **Figure 14**    Film autoradiographs (A-C) and dark-field micrographs (D-E) illustrating the expression pattern of mVRF and mRNA in the mouse. In the E14 mouse embryo (A) positive signals are present over the developing heart (Ha) and cerebral cortex (Cx). A low background signal is also present over other tissues in the section. In the E17 embryo (B) and the heart (Ha) is clearly visible due to a strong hybridisation signal. An equally strong signal is present over brown adipose tissue (Fa) in the back and around the thoracic cage. A moderate hybridisation signal is present over the spinal cord (SC) 15    and the tongue (T). The background signal is reduced compared with the E14 embryo. In the young adult mouse (C-D), positive signals are present over the heart (Ha) and adipose tissue (Fa) around the thoracic cage, while, for example, the lungs (Lu) are unlabeled. The hybridisation signal over the heart is evenly distributed over the entire left ventricle, including papillary muscles (D). In the E17 heart hybridised with an 20    excess of cold probe, no positive signal is present (E). Scale bars = 0.5 mm (A), 1.2 mm (B), 1 mm (C), 0.3 mm (D), 0.1 mm (E).

25     **Figure 15**    Dark - (A and C) and bright-field (B and D) micrographs showing mVRF mRNA expression in mouse adipose tissue (A-B) and spinal cord (C-D). A strong hybridisation signal is present over fat (A), as shown by the strong labeling in Sudan black stained sections (B). A weak signal is present also in skeletal muscle (M in A-B). In the adult spinal cord (C) the mVRF probes gave a neuronal staining pattern over the gray matter. Toluidine counterstaining showing that motoneurons in the ventral horn (D), interneurons in the deep part of the dorsal horn and around the central canal (not 30    shown) were largely positive for mVRF mRNA. Scale bars = 0.1 mm (A), 0.1 mm (B), 0.25 mm (C), 0.015 mm (D).

- 12 -

**Figure 16** Effect of VEGF on embryonic day 8 (E8) chick sensory neurons as determined by % survival, % neurite outgrowth and average neurite length ( $\mu\text{m}$ ).

**Figure 17** Effects of VEGF and SOM175 on chick glia. Tested were CNS glial,  
5 peripheral glia and CNS oligodendrocytes.

**Figure 18** Effect of various SOM175 proteins on mouse astroglial cells. ■  $^3\text{H}$  (cpm)

1. FGF-2 (10 ng/ml) positive control
2. SOM $\Delta$ X6\* 1 ng/ml
- 10 3. SOM $\Delta$ X6 10 ng/ml
4. SOM $\Delta$ X6 100 ng/ml
5. SOM $\Delta$ X6 1000 ng/ml
6. SOM $\Delta$ X6 1000 ng/ml, no heparin
7. SOMX6\*\* 1 ng/ml
- 15 8. SOMX6 10 ng/ml
9. SOMX6 100 ng/ml
10. SOMX6 1000 ng/ml
11. SOMX6 1000 ng/ml, no heparin

\* This refers to SOM175 absent exon 6;

20 \*\* This refers to SOM175.

**Figure 19** Effect of various SOM175 proteins on mouse oligodenroglial cells. ■  $^3\text{H}$  (cpm)

1. FGF-2 (10 ng/ml) positive control
- 25 2. SOM $\Delta$ X6\* 1 ng/ml
3. SOM $\Delta$ X6 10 ng/ml
4. SOM $\Delta$ X6 100 ng/ml
5. SOM $\Delta$ X6 1000 ng/ml
6. SOM $\Delta$ X6 1000 ng/ml, no heparin
- 30 7. SOMX6\*\* 1 ng/ml
8. SOMX6 10 ng/ml
9. SOMX6 100 ng/ml

- 13 -

10. SOMX6 1000 ng/ml
11. SOMX6 1000 ng/ml, no heparin
  - \* This refers to SOM175 absent exon 6;
  - \*\* This refers to SOM175.

5

**Figure 20** Effect of various SOM175 proteins on mouse forebrain neurons. ■ % survival

1. FGF-2 (10 ng/ml) positive control
  2. SOM $\Delta$ X6\* 1 ng/ml
  - 10 3. SOM $\Delta$ X6 10 ng/ml
  4. SOM $\Delta$ X6 100 ng/ml
  5. SOM $\Delta$ X6 1000 ng/ml
  6. SOM $\Delta$ X6 1000 ng/ml, no heparin
  7. SOMX6\*\* 1 ng/ml
  - 15 8. SOMX6 10 ng/ml
  9. SOMX6 100 ng/ml
  10. SOMX6 1000 ng/ml
  11. SOMX6 1000 ng/ml, no heparin
- \* This refers to SOM175 absent exon 6;  
20 \*\* This refers to SOM175.

**TABLE 1**  
**SUMMARY OF SEQUENCE IDENTITY NUMBERS**

5	SEQ ID NO:1	Nucleotide sequence of VEGF <sub>165</sub>
	SEQ ID NO:2	Amino acid sequence of VEGF <sub>165</sub>
	SEQ ID NO:3	Nucleotide sequence of SOM175 (VEGF-like molecules)
	SEQ ID NO:4	Amino acid sequence of SOM175
10	SEQ ID NO:5	Nucleotide sequence of SOM175 absent exon 6
	SEQ ID NO:6	Amino acid sequence of SOM175 absent exon 6
	SEQ ID NO:7	Nucleotide sequence of SOM175 absent exon 6 and exon 7
	SEQ ID NO:8	Amino acid sequence of SOM175 absent exon 6 and exon 7
	SEQ ID NO:9	Nucleotide sequence of SOM175 absent exon 4
15	SEQ ID NO:10	Amino acid sequence of SOM175 absent exon 4
	SEQ ID NO:11	Oligonucleotide
	SEQ ID NO:12	Oligonucleotide
	SEQ ID NO:13	Oligonucleotide
	SEQ ID NO:14	Oligonucleotide

#### EXAMPLE 1

## Human cDNA clones

The original SOM175 cDNA was isolated by screening a human foetal brain library (λzapII, Stratagene) with the cosmid D11S750 (Larsson *et al.*, 1992). The plasmid was excised "in vivo" and a single 1.1kb cDNA was obtained. Three independent SOM175 cDNAs clones were also isolated from a human foetal spleen library (Stratagene, Uni-zap) using the above-mentioned SOM175 insert as a probe. Three clones were obtained: SOM175-4A, -5A and -6A. SOM175-5A is an alternately spliced clone with exon 4 being absent (SOM175-e4). These library screens were performed using hybridisation conditions recommended by the manufacturer of the library (Stratagene) and random primed insert of SOM175.

- 15 -

Two partial human SOM175 cDNAs have also isolated from a λGT11 human melanoma cell line A2058 library (Clontech) cDNA library screens were performed using hybridisation conditions described by Church and Gilbert, 1984). In each case, the probe was generated by random priming of a PCR product derived from SOM175 (18f-  
5 700r).

#### Mouse cDNA Clones

Human SOM175 was also used to screen a mouse neonatal whole brain cDNA library (Unizap, Stratagene). Four non-chimeric clones were isolated: M175-A, B, C, D. All  
10 clones were partial cDNAs and M175-C contained several introns. Three of these cDNAs lacked the exon 6.

Another clone referred to as M1 was completely sequenced and was found to contain the full open reading frame plus part of the 5'utr and total 3'utr.  
15

#### EXAMPLE 2

#### DNA SEQUENCE ANALYSIS

The entire sequence of the cDNA clone (SOM175) was compiled and is shown in Figure 2 with its corresponding amino acid sequence. This sequence was screened for  
20 open reading frames using the MAP program (GCG, University of Wisconsin). A single open reading frame of 672bp was observed (see Figure 2). There appears to be little 5' untranslated sequences (2bp). The 3' untranslated region appears to be complete as it includes a poly-adenylation signal and poly-A tail.

25 Database homology searches were performed using the BLAST algorithm (run at NCBI, USA). This analysis revealed homology to several mammalian forms of VEGF (see Figure 3). The amount of homology between SOM175 and human VEGF<sub>165</sub> was determined using the BESTFIT program (GCG, University of Wisconsin; see Figures 4 and 5). Nucleotide homology was estimated at 69.7% and protein homology was  
30 estimated as at least 33.3% identity and 52.5% conservation using BESTFIT analysis. BLAST analysis on nucleotide sequences revealed the almost complete match to a human expressed sequence tag EST06302 (Adams *et al.*, 1993).

- 16 -

These data indicate that SOM175 encodes a growth factor that has structural similarities to VEGF. Both genes show start and stop codons in similar positions and share discrete blocks of homology. All 8 cysteines as well as a number of other VEGF residues believed to be involved in dimerisation are conserved. These residues are Cysteine-47,  
5 Proline-70, Cysteine-72, Valine-74, Arginine-77, Cysteine-78, Glycine-80, Cysteine-81, Cysteine-82, Cysteine-89, Proline-91, Cysteine-122 and Cysteine-124 and are shown in Figure 6. Given the structural conservation between VEGF and the SOM175 gene product it is also possible that they share functional similarities. It is proposed that  
10 SOM175 encodes a VEGF-like molecule that shares some properties with VEGF but has unique properties of its own. The nucleotide sequence and corresponding amino acid sequence of VEGF<sub>165</sub> is shown in Figure 1.

### EXAMPLE 3

The percentage similarity and divergence between VEGF<sub>165</sub> family and SOM175 family  
15 (protein) were analysed using the Clustal method, MegAlign Software, DNASTAR, Wisconsin. The results are shown in Tables 2.1 and 2.2. The alternatively spliced forms of SOM175 are abbreviated to SOM175-e6 where all of exon 6 is deleted; SOM175-e6 and 7 where all of exons 6 and 7 are deleted; and SOM175-e4 where all  
20 of exon 4 is deleted. The spliced form of SOM175 are shown in Figure 7. Genomic maps of SOM175 showing intron/exon boundaries are shown in Figure 8a and 8b.

- 17 -

**Table 2.1**

**A Percent nucleotide similarity between splice variants of SOM175 and  
5 human VEGF<sub>165</sub>**

	VEGF <sub>165</sub>	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
VEGF <sub>165</sub>	***	34.9	39.7	41.4	37.0
10 SOM175		***	98.9	95.1	99.2
SOM175-e6			***	98.8	84.0
SOM175-e6&7				***	80.3
SOM175-e4					***

- 18 -

**B Percent nucleotide divergence between splice variants of SOM175 and human VEGF<sub>165</sub>**

5		VEGF <sub>165</sub>	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
	<b>VEGF<sub>165</sub></b>	***	41.7	41.6	41.7	41.8
	<b>SOM175</b>		***	0.2	0.2	0.0
	<b>SOM175-e6</b>			***	0.0	0.2
10	<b>SOM175-e6&amp;7</b>				***	0.3
	<b>SOM175-e4</b>					***

**Table 2.2**

**A Percent amino acid identity between splice variants of SOM175 and human VEGF<sub>165</sub>**

15		VEGF <sub>165</sub>	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
20	<b>VEGF<sub>165</sub></b>	***	31.4	42.3	33.5	40.6
	<b>SOM175</b>		***	74.7	73.7	99.1
	<b>SOM175-e6</b>			***	76.8	99.1
	<b>SOM175-e6&amp;7</b>				***	99.1
	<b>SOM175-e4</b>					***

25

- 19 -

**B Percent amin acid divergence between splice variants f SOM175 and human VEGF<sub>165</sub>**

5		VEGF <sub>165</sub>	SOM175	SOM175-e6	SOM175-e6&7	SOM175-e4
	VEGF <sub>165</sub>	***	65.7	55.4	54.6	57.4
	SOM175		***	19.9	4.2	0.0
	SOM175-e6			***	0.0	0.0
10	SOM175-e6&7				***	0.0
	SOM175-e4					***

15

**EXAMPLE 4**

**BIOASSAYS TO DETERMINE THE FUNCTION OF SOM175**

Assays are conducted to evaluate whether SOM175 has similar activities to VEGF on endothelial cell function, angiogenesis and wound healing. Other assays are performed based on the results of receptor binding distribution studies.

20

**Assays of endothelial cell function**

*Endothelial cell proliferation.* Endothelial cell growth assays as described in Ferrara & Henzel (1989) and in Gospodarowicz *et al* (1989).

25 *Vascular permeability assay.* This assay, which utilises the Miles test in guinea pigs, will be performed as described in Miles & Miles (1952).

*Cell adhesion assay.* The influence of SOM175 on adhesion of polymorphs to endothelial cells is analysed.

30

*Chemotaxis.* This is performed using the standard Boyden chamber chemotaxis assay.

- 20 -

**Plasminogen activator assay.** Endothelial cells are tested for plasminogen activator and plasminogen activator inhibitor production upon addition of SOM175 (Pepper *et al* (1991)).

- 5   **Endothelial cell migration assay.** The ability of SOM175 to stimulate endothelial cells to migrate and form tubes is assayed as described in Montesano *et al* (1986).

**Angiogenesis Assay**

- SOM175 induction of an angiogenic response in chick chorioallantoic membrane is  
10   evaluated as described in Leung *et al* (1989).

Possible neurotrophic actions of SOM175 are assessed using the following assays:

**Neurite outgrowth assay and gene induction (PC12 cells)**

- 15   PC12 cells (a phaeochromocytoma cell line) respond to NGF and other neurotrophic factors by developing the characteristics of sympathetic neurons, including the induction of early and late genes and the extension of neurites. These cells are exposed to SOM175 and their response monitored (Drinkwater *et al* (1991); and Drinkwater *et al* (1993)).

20

**Cultured neurons from the Peripheral Nervous System (PNS)**

Primary cultures of the following PNS neurons are exposed to SOM175 and monitored for any response:

- sensory neurons from neural crest and dorsal root ganglia
- 25   - sympathetic neurons from sympathetic chain ganglia
- placode derived sensory neurons from nodose ganglia
- motoneurons from spinal cord

The assays are described in Suter *et al* (1992) and in Marinou *et al* (1992).

- 30   Where an *in vitro* response is observed, *in vivo* assays for properties such as uptake and retrograde transport are performed as described in Hendry *et al* (1992).

- 21 -

#### Nerve regeneration (PNS)

Where neurotrophic effects of SOM175 are observed, its possible role in the regeneration of axotomised sensory neurons, sympathetic neurons and motoneurons is analysed by the methods of Otto *et al* (1989); Yip *et al* (1984) and Hendry *et al* 5 (1976).

#### Actions of SOM175 on CNS neurons

The ability of SOM175 to promote survival of central nervous system neurons is analysed as described in Hagg *et al* (1992); Williams *et al* (1986); Hefti (1986) and 10 Kromer (1987).

#### Wound Healing

The ability of SOM175 to support wound healing are tested in the most clinically relevant model available, as described in Schilling *et al* (1959) and utilised by Hunt 15 *et al* (1967).

#### The Haemopoietic System

A variety of *in vitro* and *in vivo* assays on specific cell populations of the haemopoietic system are available and are outlined below:

20 *Stem Cells*

##### *Murine*

A variety of novel *in vitro* murine stem cell assays have been developed using FACS-purified cells:

25 (a) *Repopulating Stem Cells*

These are cells capable of repopulating the bone marrow of lethally irradiated mice, and have the Lin<sup>-</sup>, Rh<sup>hi</sup>, Ly-6A/E<sup>+</sup>, c-kit<sup>+</sup> phenotype. The test substance is tested on these cells either alone, or by co-incubation with multiple factors, followed by measurement of cellular proliferation by <sup>3</sup>H thymidine incorporation.

**(b) Late Stage Stem Cells**

These are cells that have comparatively little bone marrow repopulating ability but can generate D13 CFU-S. These cells have the Lin<sup>-</sup>, Rh<sup>hi</sup>, Ly-6A/E<sup>+</sup>, c-kit<sup>+</sup> phenotype. The test substance is incubated with these cells for a period of time, injected into lethally irradiated recipients, and the number of D13 spleen colonies enumerated.

**(c) Progenitor-Enriched Cells**

10 These are cells that respond *in vitro* to single growth factors, and have the Lin<sup>-</sup>, Rh<sup>hi</sup>, Ly-6A/E<sup>+</sup>, c-kit<sup>+</sup> phenotype. This assay will show if SOM175 can act directly on haemopoietic progenitor cells. The test substance is incubated with these cells in agar cultures, and the number of colonies enumerated after 7-14 days.

**15 Atherosclerosis**

Smooth muscle cells play a crucial role in the development or initiation of atherosclerosis, requiring a change in their phenotype from a contractile to a synthetic state. Macrophages, endothelial cells, T lymphocytes and platelets all play a role in the development of atherosclerotic plaques by influencing the growth and phenotypic modulations of smooth muscle cell. An *in vitro* assay that measures the proliferative rate and phenotypic modulations of smooth muscle cells in a multicellular environment is used to assess the effect of SOM175 on smooth muscle cells. The system uses a modified Rose chamber in which different cell types are seeded onto opposite coverslips.

25

**Effects of SOM175 on bone**

The ability of SOM175 to regulate proliferation of osteoblasts is assayed as described in Lowe *et al* (1991). Any effects on bone resorption are assayed as described in Lowe *et al* (1991). Effects on osteoblast migration and changes in intracellular molecules (e.g. cAMP accumulation, alkaline phosphatase levels) are analysed as described in Midy *et al* (1994).

**Effects on skeletal muscle cells**

Effects of SOM175 on proliferation of myoblasts and development of myotubes can be determined as described by Ewton *et al* (1980) and by Gospodarowicz *et al* (1976).

5

**EXAMPLE 5**  
**CLONING MURINE VEGF DNA****Isolation of cDNAs**

Murine VRF (mVRF) clones were selected from a lambda Zap new born whole brain 10 cDNA library (Stratagene). Primary phage from high density filters ( $5 \times 10^4$  pfu/plate) were identified by hybridisation with a 682bp  $^{32}\text{P}$ -labelled probe generated by PCR from an hVRF cDNA (pSOM175) as described above. Hybridisation and stringent washes of nylon membranes (Hybond-N) were carried out at 65°C under conditions described by Church and Gilbert (1984). Positive plaques were picked, 15 purified and excised *in vivo* to produce bacterial colonies containing cDNA clones in pBluescript SK-.

**Isolation of genomic clones**

Genomic clones were isolated from a mouse strain SV/129 library cloned in the 20 lambda Fix II vector (Stratagene). High density filters ( $5 \times 10^4$  pfu/filter) were screened with a 563 bp  $^{32}\text{P}$ -labelled probe generated by PCR amplification of the nucleotide 233-798 region of the mVRF cDNA (see Figure 9). Positive clones were plugged and re-screened with filters containing 400-800 pfu. Large scale phage preparations were prepared using the QIAGEN lambda kit or by ZnCl<sub>2</sub> purification 25 (Santos, 1991).

**Nucleotide sequencing and analysis**

cDNAs were sequenced on both strands using a variety of vector-based and internal primers with Applied Biosystems Incorporated (ABI) dye terminator sequencing kits 30 according to the manufacturer's specifications. Sequences were analysed on an ABI Model 373A automated DNA sequencer. Peptide homology alignments were performed using the program BESTFIT (GCG, Wisconsin).

- 24 -

#### **Identification of intron/exon boundaries**

- Identification of exon boundaries and flanking regions was carried out using PCR with mouse genomic DNA or mVRF genomic lambda clones as templates. The primers used in PCR to identify introns were derived from the hVRF sequence and to 5 allow for potential human-mouse sequence mismatches annealing temperatures 5-10°C below the estimated  $T_m$  were used. All PCR products were sized by agarose gel electrophoresis and gel purified using QIAquick spin columns (Qiagen) and the intron/exon boundaries were sequenced directly from these products. In addition, some splice junctions were sequenced from subcloned genomic fragments of MVRF.
- 10 Intron/exon boundaries were identified by comparing cDNA and genomic DNA sequences.

#### **Northern analysis**

- Total cellular RNA was prepared from a panel of fresh normal adult mouse tissues 15 (brain, kidney, liver, muscle) using the method of Chomczynski and Sacchi (1987). 20 $\mu$ g of total RNA were electrophoresed, transferred to a nylon membrane (Hybond N, Amersham) and hybridised under standard conditions (Church & Gilbert, 1984). Filters were washed at 65°C in 0.1xSSC (20xSSC is 3M NaCl/0.3M trisodium citrate), 0.1% SDS and exposed to X-ray film with intensifying screens at -70°C for 20 1-3 days.

#### **Characterisation of mVRF cDNAs**

- Murine VRF homologues were isolated by screening a murine cDNA library with an hVRF cDNA clone. Five clones of sizes varying from 0.8-1.5 kb were recovered 25 and sequenced. The cDNA sequences were complied to give a full length 1041 bp cDNA sequence covering the entire open reading frame (621 bp or 564 bp depending on the splice form, see below) and 3' UTR (379 bp), as well as 163 bp of the 5' UTR (Figure 9).
- 30 The predicted initiation codon matched the position of the start codon in hVRF. One other out of frame ATG was located at position -47 and two termination codons were observed upstream (positions -9 and -33, respectively) and in-frame with the putative

initiation codon.

The predicted N-terminal signal peptide of hVRF appears to be present in mVRF with 81% identity (17/21 amino acids). Peptide cleavage within mVRF is expected  
5 to occur after residue 21 (Figure 10). These data suggest that mature mVRF is secreted and could therefore conceivably function as a growth factor.

As with hVRF, two open reading frames (ORFs) were detected in cDNAs isolated by library screening. Four of five clones were found to be alternatively spliced and  
10 lacked a 101 bp fragment homologous to exon 6 of hVRF. The predicted peptide sequences of the two isoforms of mVRF were determined and aligned with the corresponding human isoforms (Figure 10).

15 The message encoding mVRF<sub>186</sub> contains a 621 bp ORF with coding sequences terminating at position +622, towards the end of exon 7 (Figure 9). The smaller message encoding mVRF<sub>167</sub> actually terminates downstream of the +622 TAG site due to a frame shift resulting from splicing out of the 101 bp exon 6 and the introduction of a stop codon (TGA) at position +666, near the beginning of exon 8 (Figure 9).

20 The mVRF<sub>186</sub> protein has strong homology to the amino and central portions of VEGF while the carboxyl end is completely divergent and is alanine rich. mVRF<sub>167</sub> possesses these similarities and also maintains homology to mVEGF right through to the C-terminus (Figure 11). The overall homology of mVRF<sub>167</sub> to hVRF<sub>167</sub> was  
25 85% identity and 92% similarity, respectively (Figure 10). Likewise, homology between mVRF<sub>167</sub> and mVEGF (Breier *et al*, 1992) was 49% identity and 71% conservative amino acid substitution, respectively (Figure 11).

A canonical vertebrate polyadenylation signal (AATAAA) (Birnstiel *et al*, 1986) was  
30 not present in the mVRF cDNA, however, the closely matching sequence GATAAA is present at similar positions in both mouse and human VRF cDNAs (Figure 9). In contrast to hVRF, mVRF was found to contain an AC dinucleotide repeat at the

- 26 -

extreme 3' end of the 3' UTR (nucleotide positions 998 to 1011, Figure 9).

Polymorphism of this repeat region was observed between some of the mVRF cDNAs, with the number of dinucleotides varying from 7 to 11.

## 5 Genomic characterisation of mVRF

- Intron/exon boundaries (Table 3) were mapped using primers which flanked sequences homologous to the corresponding hVRF boundaries. Introns I, III, IV and VI of mVRF (Table 3, Figure 12) were smaller than the hVRF intervening sequences. The complete genomic sequence was compiled from the 5' UTR of
- 10 mVRF through to intron VI, the largest intervening region (2.2 kb), by sequencing amplified introns and cloned genomic portions of mVRF. There was only one major difference in genomic structure between mVRF and hVRF and that was the exon 7/intron VI boundary of mVRF was located 10bp further downstream in relation to the cDNA sequence, hence exon 7 in mVRF is 10bp longer than the corresponding
- 15 exon in hVRF.

Exons 6 and 7 are contiguous in mVRF, as has been found to occur in the human homologue. The strong sequence homology between exon 6 of mVRF and hVRF (Figure 10) suggests that this sequence is not a retained intronic sequence but rather

- 20 encodes a functional part of the VRF<sub>186</sub> isoform.

- General intron/exon structure is conserved between the various members of the VEGF gene family (VEGF, PIGF, hVRF) and therefore it is not surprising that the overall genomic organisation of the mVRF gene is very similar to these genes
- 25 (Figure 12).

Previous comparative mapping studies have shown that the region surrounding the human multiple endocrine neoplasia type 1 disease locus on chromosome 11q13 is syntenic with the proximal segment of mouse chromosome 19 (Rochelle *et al*, 1992).

- 30 Since the inventors have mapped the hVRF gene to within 1kb of the human *MEN1* locus (see above) it is most likely that the murine VRF gene maps near the centromere of chromosome 19.

- 27 -

#### Expression studies of mVRF

Northern analysis of RNA from adult mouse tissues (muscle, heart, lung and liver) showed that expression appears to be ubiquitous and occurs primarily as a major band of approximately 1.3kb in size (Figure 14). This is somewhat different to the pattern observed for hVRF in which two major bands of 2.0 and 5.5 kb have been identified in all tissues examined. The 1.3 kb murine message presumably corresponds to the shorter of the human transcripts and the size variation thereof is most likely due to a difference in the length of the respective 5' UTRs.

10

#### EXAMPLE 6

#### EXPRESSION OF MURINE VEGF IN PRE- AND POST-NATAL MOUSE Animals

Timed pregnant (n=4) and young adult (n=2) mice (C57 inbred strain, ALAB, Sweden) were sacrificed with carbon dioxide, and the relevant tissues were taken out 15 and frozen on a chuck. Tissues were kept at -70°C until further use. Two gestational ages was used in this study; embryonic day 8 (E8), 14 and E17.

#### *In situ* hybridisation histochemistry

*In situ* hybridisation was performed as previously described (Dagerlind *et al*, 1992).

20 Briefly, transverse sections (14µm) were cut in a cryostat (Microm, Germany), thawed onto Probe-On slides (Fisher Scientific, USA) and stored in black sealed boxes at -70°C until used. The sequences of the synthetic 42-mer oligonucleotides complementary to mRNA encoding mVRF were

ACCACCACCTCCCTGGGCTGGCATGTGGCACGTGCATAAACG [SEQ ID

25 NO:11] (complementary to nt 120-161) and

AGTTGTTGACCACATTGCCATGAGTTCCATGCTCAGAGGC [SEQ ID

NO:12] (complementary to nt 162-203). To detect the two alternative splice forms oligonucleotide GATCCTGGGGCTGGAGTGGATGGATGATGTCAGCTGG [SEQ ID NO:13] (complementary to nt xxx-xxx) and

30 GCGGGCAGAGGATCCTGGGGCTGTCTGGCCTCACAGCACT [SEQ ID NO:14] were used. The probes were labeled at the 3'-end with deoxyadenosine-alpha[thio]triphosphate [<sup>35</sup>S] (NEN, USA) using terminal deoxynucleotidyl

transferase (IBI, USA) to a specific activity of  $7\text{-}10 \times 10^8$  cpm/ $\mu\text{g}$  and hybridised to the sections without pretreatment for 16-18 h at 42°C. The hybridisation mixture contained: 50% v/v formamide, 4 x SSC (1 x SSC = 0.15M NaCl and 0.015M sodium-citrate), 1 x Denhardt's solution (0.02% each of polyvinyl-pyrrolidone, BSA and Ficoll), 1% v/v sarcosyl (N-lauroylsarcosine; Sigma), 0.02M phosphate buffer (pH 7.0), 10% w/v dextran sulfate (Pharmacia, Sweden), 250 $\mu\text{g}/\text{ml}$  yeast tRNA (Sigma), 500 $\mu\text{g}/\text{ml}$  sheared and heat denatured salmon sperm DNA (Sigma) and 200mM dithiothreitol (DTT; LKB, Sweden). In control sections, the specificity of both probes was checked by adding a 20-fold excess of unlabeled probe to the

5 hybridisation mixture. In addition, adjacent sections were hybridised with a probe unrelated to this study which gave a different expression pattern. Following hybridisation the sections were washed several times in 1 x SSC at 55°C, dehydrated in ethanol and dipped in NTB2 nuclear track emulsion (Kodak, USA). After 3-5 weeks the sections were development in D-19 developer (Kodak, USA) and cover-

10 slipped. In some cases, sections were opposed to an autoradiographic film (Beta-max autoradiography film Amersham Ltd, UK) prior to emulsion-dipping.

15

The four different probes gave identical hybridisation patterns in all tissues examined. Mouse VRF expression was detecting already in the E8 embryo, in which positive

20 signal was recorded over structures most likely corresponding to the neuronal tube. In sagittal sections of E14 mouse embryo the strongest hybridisation signal was present over heart and in the nervous system, especially cerebral cortex (Figure 14A). A low level of expression was present in all other tissues. At a later gestational age, E17, a high mVRF mRNA signal was confined to the heart and brown fat tissue in

25 the back and around the neck (Figure 14B). Clearly positive hybridisation signals were present in the gray of the spinal cord and in the tongue (Figure 14B). Expression in the cerebral cortex was clearly reduced compared to day 14. The weak background expression seen in the E14 embryo in for example muscle, had decreased at this gestational age. A strong mVRF mRNA hybridisation signal was present

30 solely over the heart and in the brown fat in the young adult mice (Figure 14C). The signal over the heart was evenly distributed over the entire ventricular wall, including the papillary muscles (Figure 14D). In sections of heart tissue hybridised with an

excess of cold probe, no specific labeling over background signal was recorded (Figure 14E).

- Apart from the heart, mVRF mRNA signal was present over certain tissues on the
- 5 outside of the thoracic cage that morphologically resembled brown fat. This was verified with sudan black counterstaining, which showed a strong staining in the same areas (Figure 15A and 15B). In transverse sections of adult mouse spinal cord, the mVRF probes gave a neuronal staining pattern over the gray matter (Figure 15C). Counterstaining with toluidine (Figure 15D) showed that motoneurons in the ventral
- 10 horn (Figure 15C and 15D), interneurons (Figure 15C) in the deep part of the dorsal horn and around the central canal where to a large extent positive for mVRF mRNA.

#### EXAMPLE 7

#### EFFECTS OF VEGF AND SOM175 PROTEINS ON CHICK 15 SENSORY NEURONS

The effects of VEGF and SOM175 proteins on embryonic day 8 chick sensory neurons were determined using the method of Nurcombe *et al* (1992). The neuronal assay was read at 48 hours using 2000 cells per assay well. The results were obtained using  $^3\text{H}$ -thymidine counts. The percentage survival of neurons, neurite

- 20 outgrowth and average neurite length in  $\mu\text{m}$  were determined using NGF as positive control and various concentrations of VEGF, VEGF in the presence of heparin and VEGF in the presence of heparin and 5  $\mu\text{M}$ , 5'-flourouracil (5FU). 5FU kills glial cells.

- 25 The results are shown in Figure 16. The results show that VEGF is effective in promoting neuronal survival but that this requires the presence of glial cells. Figure 17 shows the results of the effect of VEGF and SOM175 on three types of chick glia. The glia tested were CNS glia, peripheral glia and CNS oligodendrocytes. Heparin was used as 10  $\mu\text{g}/\text{ml}$  in all cultures and the assay was read at 24 hours.
- 30 Results were measured in  $^3\text{H}$ -thymidine counts using 2000 cells per well.

The results show that for chick central and peripheral neurons, astroglia were markedly stimulated to proliferate by SOM175 in the presence of heparin but that chick oligodendrocytes showed negligible increase in the rate of division.

5

**EXAMPLE 8**  
**EFFECTS OF SOM175 PROTEINS ON MOUSE PRIMARY AND  
CENTRAL NEURONS**

The results in Example 7 show that VEGF isoform had an effect on chick primary  
10 and central neurons through the agency of the astrogial cells. Similar experiments  
were repeated in mouse cells.

**Culture conditions**

Neuronal and glial cells for all *in vitro* experiments were prepared and cultured  
15 according to the techniques described in "Methods in Neurosciences (Vol. 2): Cell  
Culture" Ed. P.M. Conn, Academic Press, San Diego, 1990, pp33-46 for astrogial  
cells, pp56-74 for oligodendroglial cells, and pp87-102 for central neurons.

Cells were plated onto 24-well culture clusters (Nunc) coated with poly-L-ornithine  
20 (0.1 mg/ml, 1h) at a density of 2,000 cells/well. After 48 hours in culture, neurons  
were counted in the wells under inverted phase light using well established  
techniques (Maruta *et al.* 1993) and glial cells assessed with [<sup>3</sup>H]thymidine uptake to  
monitor cell division rates as below. Heparin (10 $\mu$ g/ml, low molecular weight  
fraction, Sigma Chemical Corp.) was present at all times in the culture media except  
25 where noted. The neuronal cultures were supplemented with 5mM 5-fluoro-2-  
deoxyuridine (Sigma) to suppress background glial growth.

**<sup>3</sup>H-Thymidine incorporation assay for glial cell proliferation**

The cells were pulsed for 14h with <sup>3</sup>H-thymidine (specific activity 103  $\mu$ Ci/ $\mu$ g) from  
30 a stock concentration of 0.1 mCi/ml in standard medium, giving a final incubating  
volume of 20  $\mu$ l/well. The contents of the wells were harvested and absorbed onto  
nitrocellulose paper (Titertek, Flow). Remaining adherent cells were removed by

- 31 -

incubation with trypsin/versene (CSL Limited, Victoria, Australia) for 5 min. This procedure was carried out twice. The nitrocellulose discs were washed in a standard Titertek harvester (Flow) using first distilled water, and then methanol. The nitrocellulose discs were dried, scintillation fluid (containing 5% v/v Triton-X) added 5 and the discs counted on a scintillation counter.

Greatest activity was seen with preparations of SOM175 absent exon 6 (SOM $\Delta$ X6) on mouse astroglial cell cultures, where there was a significant stimulus to their proliferation when delivered in conjunction with heparin (Figure 16). Little stimulus 10 was given to the proliferation of oligodendroglial cells (Figure 17), and very little discernable potentiation of the survival response of isolated forebrain neurons (Figure 18). The standard deviation on all three graphs for each point was less than 8%.

The viability of neurons can be maintained by promoting glial cell proliferation. 15 Furthermore, SOM $\Delta$ X6 is a good inducer of astroglial proliferation and may be expressed in conjunction with the formation of astroglial endfeet on central nervous system endothelial cells.

Those skilled in the art will appreciate that the invention described herein is 20 susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

**TABLE 3**  
**Splice junctions of the murine VRF gene**

5	5' UTR* .....	Exon 1 >223bp	CCCAAGgtacgtgcgt	Intron I	495bp
	ttccccacagGCCCC	Exon 2 43bp	GAAAAGgtataatag	Intron II	288bp
	ctgccccacagTGGTG	Exon 3 197bp	TGCAGgtaccaggc	Intron III	196bp
	ctgagcacagATCCT	Exon 4 74bp	TGCAGgtgccagccc	Intron IV	182bp
	ctcttttcagACCTA	Exon 5 36bp	GACAGattcttggtg	Intron V	191bp
10	ctccctcctagGGTTG	Exon 6 101bp		(no intron)	
	CCCACTCCAGCCCCA	Exon 7 135bp	TGTAGgttaaggagt	Intron VI	~2200bp
	cactccccagGTGCC	Exon 8 394bp	AGAGATGGAGACACT		

Uppercase and lowercase letters denote exonic and intronic sequences respectively.

15 \* Indicates that the 5' end of exon 1 has not yet been determined.

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- 34 -

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- 36 -

## SEQUENCE LISTING

### (1) GENERAL INFORMATION:

#### (i) APPLICANT:

(countries other than US) AMRAD OPERATIONS PTY. LTD.  
(us only) Hayward, N and Weber, G

#### (ii) TITLE OF INVENTION: A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME

#### (iii) NUMBER OF SEQUENCES: 14

#### (iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: DAVIES COLLISON CAVE
- (B) STREET: 1 LITTLE COLLINS STREET
- (C) CITY: MELBOURNE
- (D) STATE: VICTORIA
- (E) COUNTRY: AUSTRALIA
- (F) ZIP: 3000

#### (v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

#### (vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: PCT INTERNATIONAL
- (B) FILING DATE: 22-FEB-1996

#### (vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: AU PN1457
- (B) FILING DATE: 02-MAR-1995
- (A) APPLICATION NUMBER: AU PN6647
- (B) FILING DATE: 20-NOV-1995
- (A) APPLICATION NUMBER: AU PN7274
- (B) FILING DATE: 22-DEC-1995

- 37 -

## (viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: HUGHES DR, E JOHN L
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## (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: +61 3 9254 2777
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## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 649 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 17...589

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCGGGCCTCC GAAACC ATG AAC TTT CTG CTG TCT TGG GTG CAT TGG AGC Met Asn Phe Leu Leu Ser Trp Val His Trp Ser 1               5               10	49
CTT GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA Leu Ala Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala 15               20               25	97
CCC ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC GAA GTG GTG AAG TTC Pro Met Ala Glu Gly Gly Gln Asn His His Glu Val Val Lys Phe 30               35               40	145
ATG GAT GTC TAT CAG CGC AGC TAC TGC CAT CCA ATC GAG ACC CTG GTG Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val 45               50               55	193
GAC ATC TTC CAG GAG TAC CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro 60               65               70               75	241
TCC TGT GTG CCC CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly 80               85               90	289

- 38 -

CTG GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met	95	100	105	337
CGG ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG ATG AGC TTC CTA Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu	110	115	120	385
CAG CAC AAC AAA TGT GAA TGC AGA CCA AAG AAA GAT AGA GCA AGA CAA Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln	125	130	135	433
GAA AAT CCC TGT GGG CCT TGC TCA GAG CGG AGA AAG CAT TTG TTT GTA Glu Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val	140	145	150	481
CAA GAT CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg	160	165	170	529
TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp	175	180	185	577
AAG CCG AGG CGG TGAGCCGGGC AGGAGGAAAGG AGCCTCCCTC AGCGTTTCGG Lys Pro Arg Arg	190			629
GAACCAGATC TCTCACCCAGG				649

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 191 amino acids
    - (B) TYPE: amino acid
    - (D) TOPOLOGY: linear
  - ii) MOLECULE TYPE: protein
  - xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asn	Phe	Leu	Leu	Ser	Trp	Val	His	Trp	Ser	Leu	Ala	Leu	Leu	Leu
1					5					10					15
Tyr	Leu	His	His	Ala	Lys	Trp	Ser	Gln	Ala	Ala	Pro	Met	Ala	Glu	Gly
					20				25					30	
Gly	Gly	Gln	Asn	His	His	Glu	Val	Val	Lys	Phe	Met	Asp	Val	Tyr	Gln
						35		40					45		
Arg	Ser	Tyr	Cys	His	Pro	Ile	Glu	Thr	Leu	Val	Asp	Ile	Phe	Gln	Glu
						50		55				60			

- 39 -

Tyr	Pro	Asp	Glu	Ile	Glu	Tyr	Ile	Phe	Lys	Pro	Ser	Cys	Val	Pro	Leu
65					70				75					80	
Met	Arg	Cys	Gly	Gly	Cys	Cys	Asn	Asp	Glu	Gly	Leu	Glu	Cys	Val	Pro
					85				90					95	
Thr	Glu	Glu	Ser	Asn	Ile	Thr	Met	Gln	Ile	Met	Arg	Ile	Lys	Pro	His
					100			105					110		
Gln	Gly	Gln	His	Ile	Gly	Glu	Met	Ser	Phe	Leu	Gln	His	Asn	Lys	Cys
					115			120					125		
Glu	Cys	Arg	Pro	Lys	Lys	Asp	Arg	Ala	Arg	Gln	Glu	Asn	Pro	Cys	Gly
					130			135					140		
Pro	Cys	Ser	Glu	Arg	Arg	Lys	His	Leu	Phe	Val	Gln	Asp	Pro	Gln	Thr
					145			150			155			160	
Cys	Lys	Cys	Ser	Cys	Lys	Asn	Thr	Asp	Ser	Arg	Cys	Lys	Ala	Arg	Gln
					165			170					175		
Leu	Glu	Leu	Asn	Glu	Arg	Thr	Cys	Arg	Cys	Asp	Lys	Pro	Arg	Arg	
					180			185					190		

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1094 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..624

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CC	ATG	AGC	CCT	CTG	CTC	CGC	CGC	CTG	CTG	CTC	GCC	GCA	CTC	CTG	CAG	47
Met	Ser	Pro	Leu	Leu	Arg	Arg	Leu	Leu	Leu	Ala	Ala	Leu	Leu	Gln		
1				5					10				15			
CTG	GCC	CCC	GCC	CAG	GCC	CCT	GTC	TCC	CAG	CCT	GAT	GCC	CCT	GGC	CAC	95
Leu	Ala	Pro	Ala	Gln	Ala	Pro	Val	Ser	Gln	Pro	Asp	Ala	Pro	Gly	His	
20									25					30		
CAG	AGG	AAA	GTG	GTG	TCA	TGG	ATA	GAT	GTG	TAT	ACT	CGC	GCT	ACC	TGC	143
Gln	Arg	Lys	Val	Val	Ser	Trp	Ile	Asp	Val	Tyr	Thr	Arg	Ala	Thr	Cys	
35									40					45		

- 40 -

CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC Gln Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr 50 55 60	191
GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly 65 70 75	239
GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His 80 85 90 95	287
CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu 100 105 110	335
GGG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys 115 120 125	383
AAA AAG GAC AGT GCT GTG AAG CCA GAC AGG GCT GCC ACT CCC CAC CAC Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His 130 135 140	431
CGT CCC CAG CCC CGT TCT GTT CCG GGC TGG GAC TCT GCC CCC GGA GCA Arg Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala 145 150 155	479
CCC TCC CCA GCT GAC ATC ACC CAT CCC ACT CCA GCC CCA GGC CCC TCT Pro Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser 160 165 170 175	527
GCC CAC GCT GCA CCC AGC ACC ACC AGC GCC CTG ACC CCC GGA CCT GCC Ala His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala 180 185 190	575
GCT GCC GCT GCC GAC GCA GCT TCC TCC GTT GCC AAG GGC GGG GCT T Ala Ala Ala Ala Asp Ala Ala Ser Ser Val Ala Lys Gly Gly Ala 195 200 205	624
AGAGCTAAC CCAGACACCT GCAGGTGCCG GAAGCTGCGA AGGTGACACA TGGCTTTCA	684
GACTCAGCAG GGTGACTTGC CTCAGAGGCT ATATCCCAGT GGGGGAACAA AGGGGAGCCT	744
GGTAAAAAAC AGCCAAGCCC CCAAGACCTC AGCCCAGGCA GAAGCTGCTC TAGGACCTGG	804
GCCTCTCAGA GGGCTTTCT GCCATCCCTT GTCTCCCTGA GGCCATCATC AAACAGGACA	864
GAGTTGGAAG AGGAGACTGG GAGGCAGCAA GAGGGGTAC ATACCAGCTC AGGGGAGAAT	924
GGAGTACTGT CTCAGTTCT AACCACTCTG TGCAAGTAAG CATCTTACAA CTGGCTCPTC	984
CTCCCCCAC TAAGAAGACC CAAACCTCTG CATAATGGGA TTTGGGCTTT GGTACAAGAA	1044
CTGTGACCCC CAACCCTGAT AAAAGAGATG GAAGGAAAAA AAAAAAAA	1094

- 41 -

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 207 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ser	Pro	Leu	Leu	Arg	Arg	Leu	Leu	Leu	Ala	Ala	Leu	Leu	Gln	Leu
1				5					10						15
Ala	Pro	Ala	Gln	Ala	Pro	Val	Ser	Gln	Pro	Asp	Ala	Pro	Gly	His	Gln
				20				25						30	
Arg	Lys	Val	Val	Ser	Trp	Ile	Asp	Val	Tyr	Thr	Arg	Ala	Thr	Cys	Gln
	35						40						45		
Pro	Arg	Glu	Val	Val	Val	Pro	Leu	Thr	Val	Glu	Leu	Met	Gly	Thr	Val
	50				55				60						
Ala	Lys	Gln	Leu	Val	Pro	Ser	Cys	Val	Thr	Val	Gln	Arg	Cys	Gly	Gly
	65				70				75				80		
Cys	Cys	Pro	Asp	Asp	Gly	Leu	Glu	Cys	Val	Pro	Thr	Gly	Gln	His	Gln
			85					90					95		
Val	Arg	Met	Gln	Ile	Leu	Met	Ile	Arg	Tyr	Pro	Ser	Ser	Gln	Leu	Gly
		100					105						110		
Glu	Met	Ser	Leu	Glu	Glu	His	Ser	Gln	Cys	Glu	Cys	Arg	Pro	Lys	Lys
	115				120							125			
Lys	Asp	Ser	Ala	Val	Lys	Pro	Asp	Arg	Ala	Ala	Thr	Pro	His	His	Arg
	130				135							140			
Pro	Gln	Pro	Arg	Ser	Val	Pro	Gly	Trp	Asp	Ser	Ala	Pro	Gly	Ala	Pro
145					150				155				160		
Ser	Pro	Ala	Asp	Ile	Thr	His	Pro	Thr	Pro	Ala	Pro	Gly	Pro	Ser	Ala
			165					170					175		
His	Ala	Ala	Pro	Ser	Thr	Thr	Ser	Ala	Leu	Thr	Pro	Gly	Pro	Ala	Ala
			180				185					190			
Ala	Ala	Ala	Asp	Ala	Ala	Ser	Ser	Val	Ala	Lys	Gly	Gly	Ala		
			195				200					205			

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 993 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) **FEATURE:**

(A) NAME/KEY: CDS  
(B) LOCATION: 3 . . 566

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5.

CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln	1	5	10	15	47
CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His	20	25	30		95
CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys	35	40	45		143
CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC Gln Pro Arg Glu Val Val Pro Leu Thr Val Glu Leu Met Gly Thr	50	55	60		191
GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly	65	70	75		239
GC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC ly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His	80	85	90	95	287
AA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG In Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu	100	105	110		335
GG GAG ATG TCC CTG GAA GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA ly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys	115	120	125		383
AA AAG GAC AGT GCT GTG AAG CCA GAT AGC CCC AGG CCC CTC TGC CCA rs Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro	130	135	140		431

- 43 -

CGC TGC ACC CAG CAC CAC CAG CGC CCT GAC CCC CGG ACC TGC CGC TGC Arg Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys 145 150 155	479
CGC TGC CGA CGC CGC AGC TTC CTC CGT TGC CAA GGG CGG GGC TTA GAG Arg Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu 160 165 170 175	527
CTC AAC CCA GAC ACC TGC AGG TGC CGG AAG CTG CGA AGG TGACACATGG Leu Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg 180 185	576
CTTTTCAGAC TCAGCAGGGT GACTTGCTC AGAGGCTATA TCCCAGTGGG GGAACAAAGG GGAGCCTGGT AAAAAACAGC CAAGCCCCA AGACCTCAGC CCAGGCAGAA GCTGCTCTAG GACCTGGGCC TCTCAGAGGG CTCTTCTGCC ATCCCTTGTC TCCCTGAGGC CATCATCAA CAGGACAGAG TTGGAAGAGG AGACTGGGAG GCAGCAAGAG GGGTCACATA CCAGCTCAGG GGAGAATGGA GTACTGTCTC AGTTTCTAAC CACTCTGTGC AAGTAAGCAT CTTACAAC TG GCTCTTCCTC CCCTCACTAA GAAGACCAA ACCTCTGCAT AATGGGATTG GGGCTTGGT ACAAGAACTG TGACCCCCAA CCCTGATAAA AGAGATGGAA GGAAAAAAA AAAAAAA	636 696 756 816 876 936 993

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 188 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu 1 5 10 15
Ala Pro Ala Gin Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln 20 25 30
Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln 35 40 45
Pro Arg Glu Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val 50 55 60
Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly 65 70 75 80

- 44 -

Cys	Cys	Pro	Asp	Asp	Gly	Leu	Glu	Cys	Val	Pro	Thr	Gly	Gln	His	Gln
					85				90						95
Val	Arg	Met	Gln	Ile	Leu	Met	Ile	Arg	Tyr	Pro	Ser	Ser	Gln	Leu	Gly
					100			105						110	
Glu	Met	Ser	Leu	Glu	Glu	His	Ser	Gln	Cys	Glu	Cys	Arg	Pro	Lys	Lys
					115			120						125	
Lys	Asp	Ser	Ala	Val	Lys	Pro	Asp	Ser	Pro	Arg	Pro	Leu	Cys	Pro	Arg
					130			135						140	
Cys	Thr	Gln	His	His	Gln	Arg	Pro	Asp	Pro	Arg	Thr	Cys	Arg	Cys	Arg
					145			150			155			160	
Cys	Arg	Arg	Arg	Ser	Phe	Leu	Arg	Cys	Gln	Gly	Arg	Gly	Leu	Glu	Leu
					165			170						175	
Asn	Pro	Asp	Thr	Cys	Arg	Cys	Arg	Lys	Leu	Arg	Arg				
					180			185							

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 858 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..431

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CC	ATG	AGC	CCT	CTG	CTC	CGC	CGC	CTG	CTG	CTG	GCC	GCA	CTC	CTG	CAG	47
Met	Ser	Pro	Leu	Leu	Arg	Arg	Leu	Leu	Leu	Ala	Ala	Leu	Leu	Gln		
1				5					10					15		
CTG	GCC	CCC	GCC	CAG	GCC	CCT	GTC	TCC	CAG	CCT	GAT	GCC	CCT	GGC	CAC	95
Leu	Ala	Pro	Ala	Gln	Ala	Pro	Val	Ser	Gln	Pro	Asp	Ala	Pro	Gly	His	
							20		25					30		
CAG	AGG	AAA	GTG	GTG	TCA	TGG	ATA	GAT	GTG	TAT	ACT	CGC	GCT	ACC	TGC	143
Gln	Arg	Lys	Val	Val	Ser	Trp	Ile	Asp	Val	Tyr	Thr	Arg	Ala	Thr	Cys	
							35		40					45		
CAG	CCC	CGG	GAG	GTG	GTG	CCC	TTG	ACT	GTG	GAG	CTC	ATG	GGC	ACC		191
Gln	Pro	Arg	Glu	Val	Val	Val	Pro	Leu	Thr	Val	Glu	Leu	Met	Gly	Thr	
							50		55					60		

- 45 -

GTC GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly 65 70 75	239
GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His 80 85 90 95	287
CAA GTC CGG ATG CAG ATC CTC ATG ATC CGG TAC CCG AGC AGT CAG CTG Gln Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu 100 105 110	335
GGG GAG ATG TCC CTG GAA CAC AGC CAG TGT GAA TGC AGA CCT AAA Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys 115 120 125	383
AAA AAG GAC AGT GCT GTG AAG CCA GAT AGG TGC CGG AAG CTG CGA AGG Lys Lys Asp Ser Ala Val Lys Pro Asp Arg Cys Arg Lys Leu Arg Arg 130 135 140	431
TGACACATGG CTTTCAGAC TCAGCAGGGT GACTTGCCTC AGAGGCTATA TCCCAGTGGG GGAACAAAGG GGAGCCTGGT AAAAAACAGC CAAGCCCCA AGACCTCAGC CCAGGCAGAA GCTGCTCTAG GACCTGGGCC TCTCAGAGGG CTCTTCTGCC ATCCCTTGTC TCCCTGAGGC CATCATCAA CAGGACAGAG TTGGAAGAGG AGACTGGAG GCAGCAAGAG GGGTCACATA CCAGCTCAGG GGAGAATGGA GTACTGTCTC AGTTTCTAAC CACTCTGTGC AAGTAAGCAT CTTACAAC TG GCTCTTCCTC CCCTCACTAA GAAGACCCAA ACCTCTGCAT AATGGGATTT GGGCTTTGGT ACAAGAACTG TGACCCCCAA CCCTGATAAA AGAGATGGAA GGAAAAAAA AAAAAAA	491 551 611 671 731 791 851 858

- 46 -

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 143 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Ser	Pro	Leu	Leu	Arg	Arg	Leu	Leu	Leu	Ala	Ala	Leu	Leu	Gln	Leu
1				5					10						15
Ala	Pro	Ala	Gln	Ala	Pro	Val	Ser	Gln	Pro	Asp	Ala	Pro	Gly	His	Gln
					20				25					30	
Arg	Lys	Val	Val	Ser	Trp	Ile	Asp	Val	Tyr	Thr	Arg	Ala	Thr	Cys	Gln
					35			40					45		
Pro	Arg	Glu	Val	Val	Val	Pro	Leu	Thr	Val	Glu	Leu	Met	Gly	Thr	Val
					50			55				60			
Ala	Lys	Gln	Leu	Val	Pro	Ser	Cys	Val	Thr	Val	Gln	Arg	Cys	Gly	Gly
					65		70			75				80	
Cys	Cys	Pro	Asp	Asp	Gly	Leu	Glu	Cys	Val	Pro	Thr	Gly	Gln	His	Gln
					85				90				95		
Val	Arg	Met	Gln	Ile	Leu	Met	Ile	Arg	Tyr	Pro	Ser	Ser	Gln	Leu	Gly
					100			105					110		
Glu	Met	Ser	Leu	Glu	Glu	His	Ser	Gln	Cys	Glu	Cys	Arg	Pro	Lys	Lys
					115			120				125			
Lys	Asp	Ser	Ala	Val	Lys	Pro	Asp	Arg	Cys	Arg	Lys	Leu	Arg	Arg	
					130			135				140			

- 47 -

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 910 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..305

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CC ATG AGC CCT CTG CTC CGC CGC CTG CTG CTC GCC GCA CTC CTG CAG Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln	47
1 5 10 15	
CTG GCC CCC GCC CAG GCC CCT GTC TCC CAG CCT GAT GCC CCT GGC CAC Leu Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His	95
20 25 30	
CAG AGG AAA GTG GTG TCA TGG ATA GAT GTG TAT ACT CGC GCT ACC TGC Gln Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys	143
35 40 45	
CAG CCC CGG GAG GTG GTG GTG CCC TTG ACT GTG GAG CTC ATG GGC ACC Gln Pro Arg Glu Val Val Pro Leu Thr Val Glu Leu Met Gly Thr	191
50 55 60	
GTG GCC AAA CAG CTG GTG CCC AGC TGC GTG ACT GTG CAG CGC TGT GGT Val Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly	239
65 70 75	
GGC TGC TGC CCT GAC GAT GGC CTG GAG TGT GTG CCC ACT GGG CAG CAC Gly Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His	287
80 85 90 95	
CAA GTC CGG ATG CAG ACC TAAAAAAAAG GACAGTGCTG TGAAGCCAGA Gln Val Arg Met Gln Thr	335
100	
CAGGGCTGCC ACTCCCCACC ACCGTCCCCA GCCCGTTCT GTTCCGGGCT GGGACTCTGC	395
CCCCGGAGCA CCCTCCCCAG CTGACATCAC CCATCCACT CCAGCCCCAG GCCCTCTGC	455
CCACGCTGCA CCCAGCACCA CCAGGCCCT GACCCCCGGA CCTGCCGCTG CCGCTGCCGA	515
CGCCGCAGCT TCCTCCGTTG CCAAGGGCGG GGCTTAGAGC TCAACCCAGA CACCTGCAGG	575
TGCCGGAAGC TGCGAAGGTG ACACATGGCT TTTCAGACTC AGCAGGGTGA CTTGCCTCAG	635
AGGCTATATC CCAGTGGGA ACAAAAGAGGA GCCTGGTAAA AAACAGCCAA GCCCCCAAGA	695

- 48 -

CCTCAGCCCA GGCAGAACGCT GCTCTAGGAC CTGGGCCTCT CAGAGGGCTC TTCTGCCATC	755
CCTTGTCTCC CTGAGGCCAT CATCAAACAG GACAGAGTTG GAAGAGGAGA CTGGGAGGCA	815
GCAAGAGGGG TCACATACCA GCTCAGGGGA GAATGGAGTA CTGTCTCAGT TTCTAACAC	875
TCTGTGCAAG TAAGCATCTT ACAACTGGCT CTTCC	910

## (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 101 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Ala Leu Leu Gln Leu			
1	5	10	15
Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln			
20	25	30	
Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln			
35	40	45	
Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val			
50	55	60	
Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly			
65	70	75	80
Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln			
85	90	95	
Val Arg Met Gln Thr			
100			

- 49 -

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACCACCACCT CCCTGGGCTG GCATGTGGCA CGTGCATAAA CG

42

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGTTGTTTGA CCACATTGCC CATGAGTTCC ATGCTCAGAG GC

42

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATCCTGGGG CTGGAGTGGG ATGGATGATG TCAGCTGG

38

- 50 -

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCAGGGCAGAG GATCCTGGGG CTGTCTGGCC TCACAGCACT

40

## CLAIMS:

1. A biologically isolated proteinaceous molecule having the following characteristics:
  - (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF).
2. A proteinaceous molecule according to claim 1 wherein the molecule exhibits at least one of the following properties:
  - (i) an ability to induce vascular endothelial cells;
  - (ii) an ability to interact with *flt-1/flk-1* family of receptors; and/or
  - (iii) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.
3. A proteinaceous molecule according to claim 1 or 2 wherein said molecule has the capacity to induce astroglial proliferation.
4. A proteinaceous molecule according to claim 1 wherein said molecule is of human origin.
5. A proteinaceous molecule according to claim 1 wherein said molecule is of non-human origin.
6. A proteinaceous molecule according to claim 5 wherein said molecule is of livestock animal, companion animal, laboratory test animal, avian, fish or reptilian origin.
7. A proteinaceous molecule according to claim 5 wherein said molecule is encoded by a gene located at chromosome 11q13.

- 52 -

8. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 30%.
9. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 40%.
10. A proteinaceous molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 60-70%.
11. A proteinaceous molecule according to claim 1 comprising a sequence of amino acids as set forth in SEQ ID NO:4 or a part, fragment, derivative or analogue thereof.
12. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:6 or a part, fragment, derivative or analogue thereof.
13. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:8 or a part, fragment, derivative or analogue thereof.
14. A proteinaceous molecule according to claim 1 comprising an amino acid sequence substantially set forth in SEQ ID NO:10 or a part, fragment, derivative or analogue thereof.
15. A recombinant molecule having the following characteristics:
  - (i) an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one biological property in common with VEGF.

16. A recombinant molecule having the following characteristics:
  - (i) an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one biological property in common with VEGF.
17. A recombinant molecule having the following characteristics:
  - (i) an amino acid sequence substantially as set forth in SEQ ID NO:8 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one biological property in common with VEGF.
18. A recombinant molecule having the following characteristics:
  - (i) an amino acid sequence substantially as set forth in SEQ ID NO:10 or having at least about 15% similarity to but at least about 5% dissimilarity to the amino acid sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one biological property in common with VEGF.
19. A recombinant molecule according to claim 15 or 16 or 17 or 18 having at least one of the following properties:
  - (a) an ability to induce vascular endothelial cells;
  - (b) an ability to interact with *flt1/flki* family of receptors;
  - (c) an ability to induce cell migration, cell survival and/or increase intracellular levels of alkaline phosphatase.
20. A recombinant molecule according to claim 15 or 16 or 17 or 18 having the capacity to induce astroglial proliferation.

- 54 -

21. A recombinant molecule according to claim 20 wherein the molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6.
22. A peptide fragment corresponding to a portion of the amino acid sequence set forth in SEQ ID NO:4 or a derivative or chemical equivalent thereof.
23. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:6 or a chemical equivalent thereof.
24. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:8 or a chemical equivalent thereof.
25. A peptide fragment according to claim 22 having the sequence set forth in SEQ ID NO:10 or a chemical equivalent thereof.
26. A nucleic acid molecule comprising a sequence of nucleotides or complementary to a sequence encoding a proteinaceous molecule having the following characteristics:
  - (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF).
27. A nucleic acid molecule according to claim 26 wherein the proteinaceous molecule exhibits at least one of the following properties:
  - (i) an ability to induce vascular endothelial cells;
  - (ii) an ability to interact with *flt-1/flk-1* family of receptors; and/or
  - (iii) an ability to induce cell migration, cell survival and/or an increase in intracellular levels of alkaline phosphatase.
28. A nucleic acid molecule according to claim 27 wherein the proteinaceous molecule has the capacity to induce astrogial proliferation.

- 55 -

29. A nucleic acid molecule according to claim 28 wherein said molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:6.
30. A nucleic acid molecule according to claim 1 wherein said molecule is of human origin.
31. A nucleic acid molecule according to claim 1 wherein the percentage similarity to SEQ ID NO:2 is at least about 30%.
32. A nucleic acid molecule according to claim 26 comprising a nucleotide sequence substantially as set forth in SEQ ID NO:3 or having at least 15% similarity thereto or capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the nucleotide sequence has at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence set forth in SEQ ID NO:3.
33. A nucleic acid molecule according to claim 26 encoding a murine homologue of human VEGF and comprising a nucleotide sequence substantially as set forth in Figure 9.
34. A pharmaceutical composition comprising a proteinaceous molecule according to claim 1 or 2 or 3 or 11 and one or more pharmaceutically acceptable carriers and/or diluents.
35. A method for preparing a recombinant molecule having the following characteristics:
- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
  - (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),
- said method comprising expressing a nucleic acid molecule encoding said recombinant

- 56 -

molecule by a suitable host grown under conditions effective to synthesise said recombinant molecule and then isolating said molecule.

36. A method according to claim 35 wherein the nucleic acid molecule comprises a sequence of nucleotides as set forth in SEQ ID NO:3 or having at least 15% similarity thereto or is capable of hybridising under low stringency conditions to a reverse complement of the nucleotide sequence as set forth in SEQ ID NO:3 provided that the nucleotide sequence has at least 15% similarity but at least 30% dissimilarity to the nucleotide sequence set forth in SEQ ID NO:3.

37. A method of inducing astrogial proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astrogial proliferation.

38. A method according to claim 37 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:3 or is a derivative thereof.

39. A method according to claim 37 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or is a derivative thereof.

40. A method of promoting neuronal survival and/or proliferation in a mammal, said method comprising administering to said mammal an effective amount of a recombinant proteinaceous molecule having the characteristics:

- 57 -

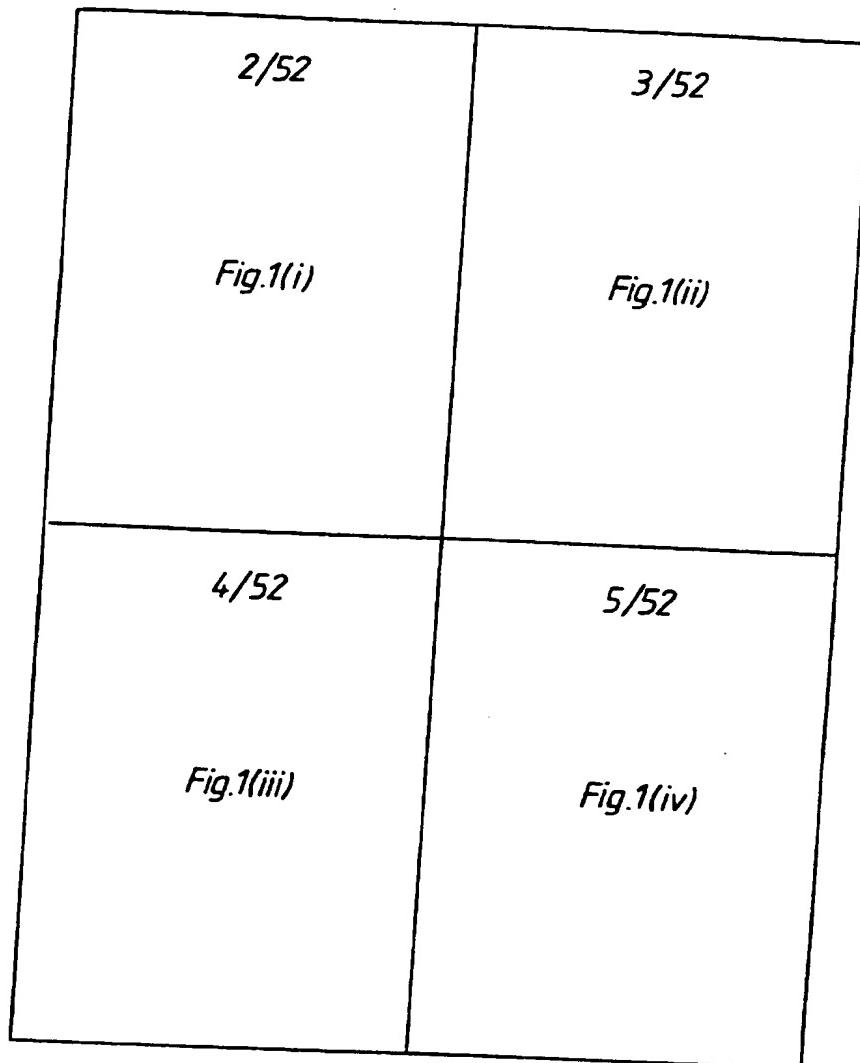
- (i) comprises an amino acid sequence having at least about 15% similarity but at least about 5% dissimilarity to the sequence set forth in SEQ ID NO:2;
- (ii) exhibits at least one property in common with vascular endothelial growth factor (VEGF),

said administration being for a time and under conditions sufficient to induce astroglial proliferation.

41. A method according to claim 40 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:3 or is a derivative thereof.

41. A method according to claim 40 wherein the recombinant proteinaceous molecule comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or is a derivative thereof.

1/52



2/52

1	TCGGCCTCC GAAACC ATG AAC TTT CTG Met Asn Phe Leu 1
50	CTT GCC TTG CTG CTC TAC CTC CAC Leu Ala Leu Leu Leu Tyr Leu His 15
98	CCC ATG GCA GAA GGA GGA GGG CAG Pro Met Ala Glu Gly Gly Gly Gln 30 35
146	ATG GAT GTC TAT CAG CGC AGC TAC Met Asp Val Tyr Gln Arg Ser Tyr 45 50
194	GAC ATC TTC CAG GAG TAC CCT GAT Asp Ile Phe Gln Glu Tyr Pro Asp 60 65
242	TCC TGT GTG CCC CTG ATG CGA TGC Ser Cys Val Pro Leu Met Arg Cys 80
290	CTC GAG TGT GTG CCC ACT GAG GAG Leu Glu Cys Val Pro Thr Glu Glu 95
338	CGG ATC AAA CCT CAC CAA GGC CAG Arg Ily Lys Pro His Gln Gly Gln 110 115

*Fig.1(i)*

3/52

CTG TCT TGG GTG CAT TGG AGC Leu Ser Trp Val His Trp Ser 5 10	49
CAT GCC AAG TGG TCC CAG GCT GCA His Ala Lys Trp Ser Gln Ala Ala 20 25	97
AAT CAT CAC GAA GTG GTG AAG TTC Asn His His Glu Val Val Lys Phe 40	145
TGC CAT CCA ATC GAG ACC CTG GTG Cys His Pro Ile Glu Thr Leu Val 55	193
GAG ATC GAG TAC ATC TTC AAG CCA Glu Ile Glu Tyr Ile Phe Lys Pro 70 75	241
GGG GGC TGC TGC AAT GAC GAG GGC Gly Gly Cys Cys Asn Asp Glu Gly 85 90	289
TCC AAC ATC ACC ATG CAG ATT ATG Ser Asn Ile Thr Met Gln Ile Met 100 105	337
CAC ATA GGA GAG ATG AGC TTC CTA His Ile Gly Glu Met Ser Phe Leu 120	385

*Fig.1(ii)*

4/52

386	CAG CAC AAC AAA TGT GAA TGC AGA Gln His Asn Lys Cys Glu Cys Arg 125 130
434	GAA AAT CCC TGT GGG CCT TGC TCA Glu Asn Pro Cys Gly Pro Cys Ser 140 145
482	CAA GAT CCG CAG ACG TGT AAA TGT Gln Asp Pro Gln Thr Cys Lys Cys 160
530	TGC AAG GCG AGG CAG CTT GAG TTA Cys Lys Ala Arg Gln Leu Glu Leu 175
578	AAG CCG AGG CGG TGAGCCGGGC AGGAG Lys Pro Arg Arg 190
630	GAACCAGATC TCTCACCAAGG

*Fig. 1(iii)*

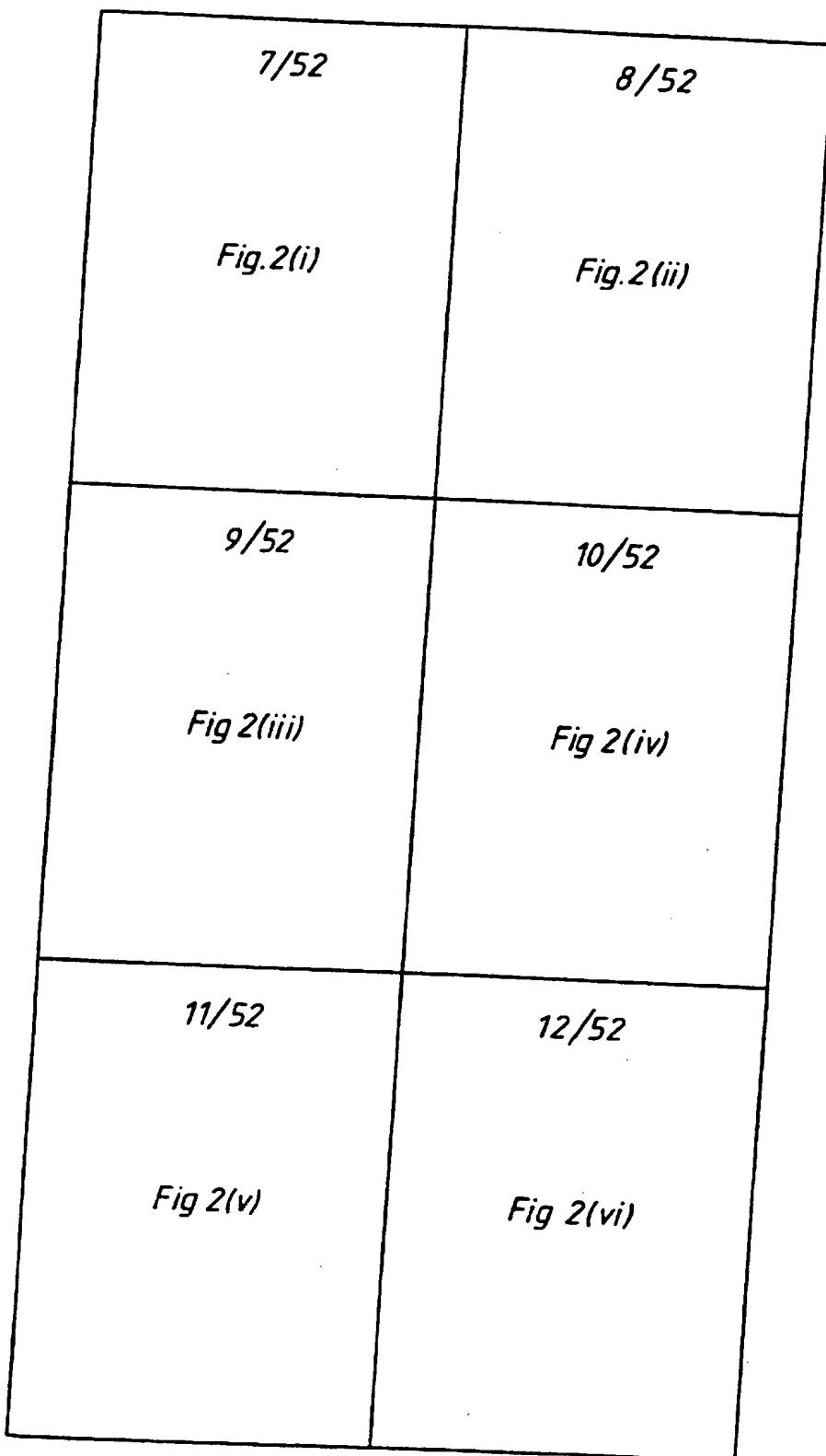
5/52

CCA AAG AAA GAT AGA GCA AGA CAA	433
Pro Lys Lys Asp Arg Ala Arg Gln	
135	
GAG CGG AGA AAG CAT TTG TTT GTA	481
Glu Arg Arg Lys His Leu Phe Val	
150	155
TCC TGC AAA AAC ACA GAC TCG CGT	529
Ser Cys Lys Asn Thr Asp Ser Arg	
165	170
AAC GAA CGT ACT TGC AGA TGT GAC	577
Asn Glu Arg Thr Cys Arg Cys Asp	
180	185
GAAGG AGCCTCCCTC AGCGTTTCGG	629

649

*Fig. 1(iv)*

6/52



7/52

1	CC ATG AGC CCT CTG CTC CGC CGC Met Ser Pro Leu Leu Arg Arg 1                                       5
48	CTG GCC CCC GCC CAG GCC CCT GTC Leu Ala Pro Ala Gln Ala Pro Val 20
96	CAG AGG AAA GTG GTG TCA TGG ATA Gln Arg Lys Val Val Ser Trp Ile 35
144	CAG CCC CGG GAG GTG GTG GTG CCC Gln Pro Arg Glu Val Val Val Pro 50                                   55
192	GTG GCC AAA CAG CTG GTG CCC AGC Val Ala Lys Gln Leu Val Pro Ser 65                                   70
240	GGC TGC TGC CCT GAC GAT GGC CTG Gly Cys Cys Pro Asp Asp Gly Leu 80                                   85
288	CAA GTC CGG ATG CAG ATC CTC ATG Gln Val Arg Met Gln Ile Leu Met 100
336	GGG GAG ATG TCC CTG GAA GAA CAC Gly Glu Met Ser Leu Glu Glu His 115

Fig.2(i)

8/52

CTG CTG CTC GCC GCA CTC CTG CAG Leu Leu Leu Ala Ala Leu Leu Gln 10 15	47
TCC CAG CCT GAT GCC CCT GGC CAC Ser Gln Pro Asp Ala Pro Gly His 25 30	95
GAT GTG TAT ACT CGC GCT ACC TGC Asp Val Tyr Thr Arg Ala Thr Cys 40 45	143
TTG ACT GTG GAG CTC ATG GGC ACC Leu Thr Val Glu Leu Met Gly Thr 60	191
TGC GTG ACT GTG CAG CGC TGT GGT Cys Val Thr Val Gln Arg Cys Gly 75	239
GAG TGT GTG CCC ACT GGG CAG CAC Glu Cys Val Pro Thr Gly Gln His 90 95	287
ATC CGG TAC CCG AGC AGT CAG CTG Ile Arg Tyr Pro Ser Ser Gln Leu 105 110	335
AGC CAG TGT GAA TGC AGA CCT AAA Ser Gln Cys Glu Cys Arg Pro Lys 120 125	383

*Fig. 2(ii)*

9/52

384	AAA AAG GAC AGT GCT GTG AAG CCA Lys Lys Asp Ser Ala Val Lys Pro 130	135
432	CGT CCC CAG CCC CGT TCT GTT CCG Arg Pro Gln Pro Arg Ser Val Pro 145	150
480	CCC TCC CCA GCT GAC ATC ACC CAT Pro Ser Pro Ala Asp Ile Thr His 160	165
528	GCC CAC GCT GCA CCC AGC ACC ACC Ala His Ala Ala Pro Ser Thr Thr 180	
576	GCT GCC GCT GCC GAC GCC GCA GCT Ala Ala Ala Ala Asp Ala Ala Ala 195	

*Fig. 2(iii)*

10/52

GAC	AGG	GCT	GCC	ACT	CCC	CAC	CAC		431
Asp	Arg	Ala	Ala	Thr	Pro	His	His		
				140					
GGC	TGG	GAC	TCT	GCC	CCC	GGA	GCA		479
Gly	Trp	Asp	Ser	Ala	Pro	Gly	Ala		
			155						
CCC	ACT	CCA	GCC	CCA	GGC	CCC	TCT		527
Pro	Thr	Pro	Ala	Pro	Gly	Pro	Ser		
	170					175			
AGC	GCC	CTG	ACC	CCC	GGA	CCT	GCC		575
Ser	Ala	Leu	Thr	Pro	Gly	Pro	Ala		
	185				190				
TCC	TCC	GTT	GCC	AAG	GGC	GGG	GCT	T	624
Ser	Ser	Val	Ala	Lys	Gly	Gly	Gly	Ala	
	200				205				

Fig.2(iv)

11/52

625	AGAGCTCAAC CCAGACACCT GCAGGGTGC CG
685	GACTCAGCAG GGTGACTTGC CTCAGAGGCT
745	GGTAAAAAAC AGCCAAGCCC CCAAGACCTC
805	GCCTCTCAGA GGGCTCTTCT GCCATCCCTT
865	GAGTTGGAAG AGGAGACTGG GAGGCAGCAA
825	GGAGTACTGT CTCAGTTCT AACCACTCTG
985	CTCCCCTCAC TAAGAAGACC CAAACCTCTG
1045	CTGTGACCCCC CAACCCTGAT AAAAGAGATG

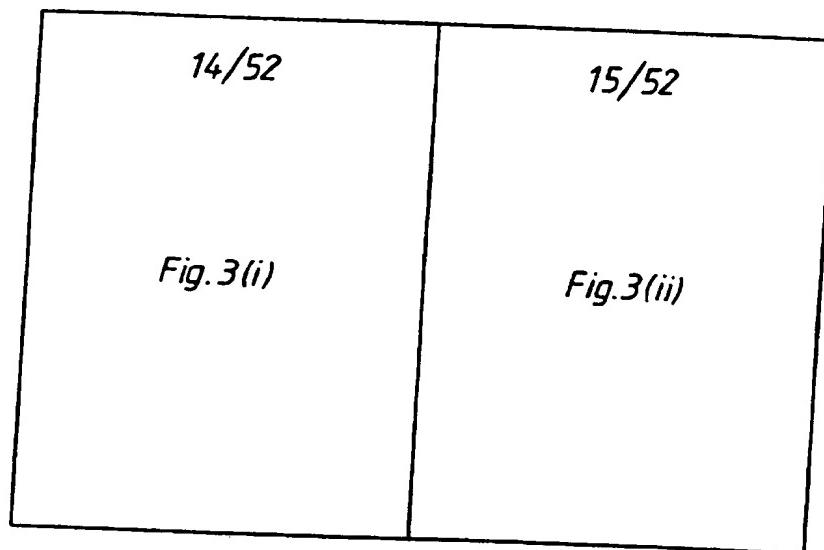
*Fig. 2(v)*

12/52

GAAGCTGCGA AGGTGACACA TGGCTTTCA	684
ATATCCCAGT GGGGAAACAA AGGGGAGCCT	744
AGCCCAGGCA GAAGCTGCTC TAGGACCTGG	804
GTCTCCCTGA GGCCATCATC AACACAGGACA	864
GAGGGGTCAC ATACCAGCTC AGGGGAGAAT	924
TGCAAGTAAG CATCTTACAA CTGGCTCTTC	984
CATAATGGGA TTTGGGCTTT GGTACAAGAA	1044
GAAGGAAAAA AAAAAAAAAAA	1094

*Fig. 2(vi)*

13/52



14/52

>VEGF\_HUMAN VEGF\_HUMAN VASCULAR ENDOTHELIAL  
 (VASCULAR 215 AA.  
 LENGTH = 215

SCORE = 181 (92.4 BITS), EXPECT = 6.4e-20,  
 IDENTITIES = 33/75 (44%), POSITIVES = 48/75

QUERY: 31 HQRKVVSWIDVYTRATCQPREGVVVPLTVEL  
 +++ VV +DVY R+ C+P E +V + E

SBJCT: 36 NHHEVVKFMDVYQRSYCHPIETLVDIFQEY

QUERY: 91 PTGQHQVRMQILMIR 105  
 PT + + MQI+ I+

SBJCT: 96 PTEESNITMQIMRIK 110

SCORE = 76 (38.8 BITS), EXPECT = 0.0011,  
 IDENTITIES = 12/19 (63%), POSITIVES = 16/19

QUERY: 110 QLGEMSLEEHSQCECRPKK 128  
 ++GEMS +H+ CECRPKK

SBJCT: 116 HIGEMSFLQHNKCECRPKK 134

SCORE = 72 (36.8 BITS), EXPECT = 0.0046,  
 IDENTITIES = 14/21 (66%), POSITIVES = 15/21

QUERY: 202 RCQGRGLELNPDTCRCKLRR 222  
 RC +R LEVN TCRC K RR

SBJCT: 195 RCKARQLELNERTCRCDKP RR 215

SCORE = 46 (23.5 BITS), EXPECT = 47.,  
 IDENTITIES = 6/10 (60%), POSITIVES = 9/10

QUERY: 187 DPRTCRCKCR 196  
 DP+TC+C C+

SBJCT: 181 DPQTCKCSCK 190  
 SUBSTITUTE SHEET (RULE 26)

Fig.3(i)

15/52

## GROWTH FACTOR PRECURSOR (VEGF)

P =  $6.4 \times 10^{-20}$   
(64%)

MGTVAKQLVPSCVTVQRCGGCCPDDGLECV 90  
+ PSCV + RCGGCC D+GLECV  
PDEIEYIFKPSCVPLMRCGGCCNDEGLECV 95

POISSON P(2) =  $9.1 \times 10^{-12}$   
(84%)

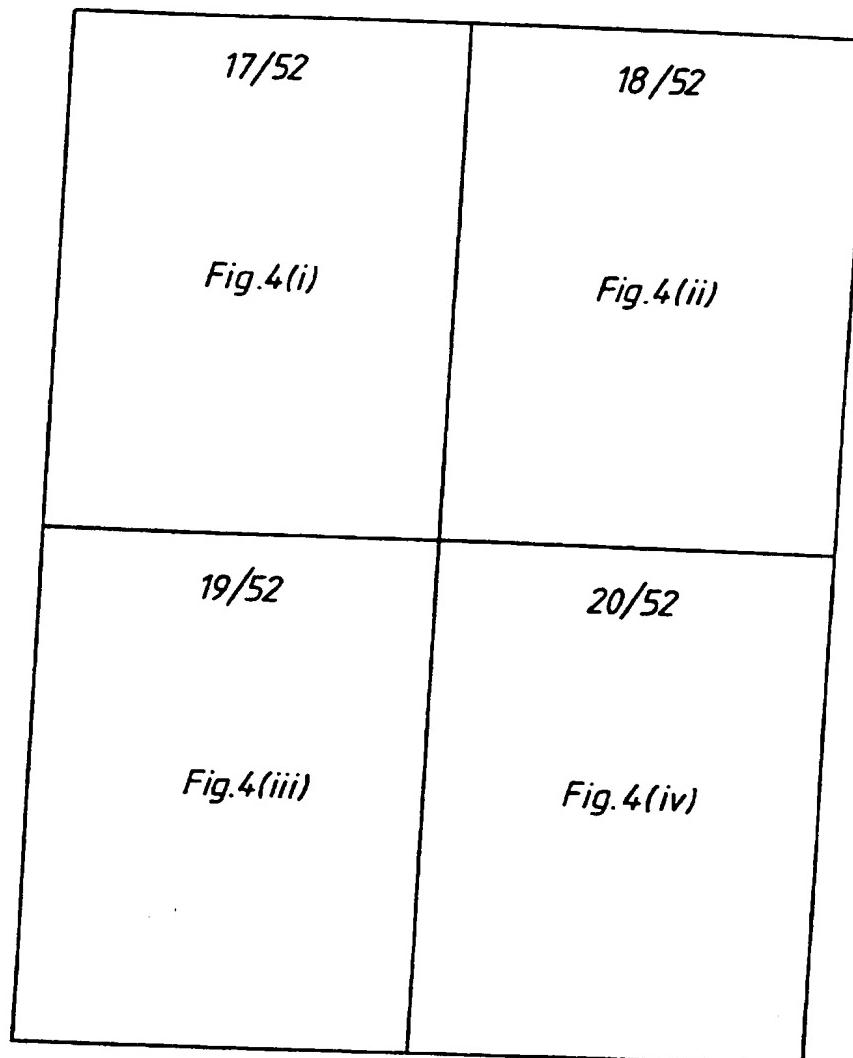
POISSON P(3) =  $3.6 \times 10^{-18}$   
(71%)

POISSON P(4) =  $7.3 \times 10^{-10}$   
(90%)

*Fig. 3(i)*

SUBSTITUTE SHEET (RULE 26)

16/52



17/52

Gap Weight: 3.00	Average Match: 1.000
Length Weight: 0.100	Average Mismatch: -0.900
Quality: 100.9	Length: 739
Ratio: 0.175	Gaps: 30
Percent	Percent
Similarity: 69.703	Identity: 69.703

28	ATGAGCCCTCTGCTCCGCCGCCTGC 
17	ATGAACTTTCTGCT....GTCT..
68	TGCAGCTGGCCCCCGCCCAGGCC 
57	TGCTGCTCTACCTCCACCATGCCAA
118	CACCAGAGGA..... 
106	AGAAGGAGGAGGGCAGAATCATCAC
140	GTGTATACTCGC.GCTACCTGCCAG 
152	GTCTATCAGCGCAGCTA.CTGCCAT
194	T....GA....CTGTGGAGCTCAT 
201	TCCAGGAGTACCCCTGATGAGATCGA
235	CCCAGCTGCGTGACTGTGCAGCGCT 
239	CCATCCTGTGTGCCCTGATGCGAT
285	CCTGGAGTGTGTGCCCACTGGGCAG 
289	CCTGGAGTGTGTGCCCACTGAGGAG

Fig. 4(i)

18/52

*Fig.4(ii)*  
SUBSTITUTE SHEET (RULE 26)

19/52

330	.....CCTCATGATCCGGTACC
339	GGATCAAACCTCA.....C
369	GTCCCTGGAAGAACACAGCCAGTGT
376	GAGCTTCCTACAGCACAAACAAATGT
419	GTGCTGTGAAGCCAGACAGGGCTGC
423	G.....AGCAAGACAAG.....
469	CGTTCTGTTCCGGGCTGGGACTCTG
443	...TGTGGGCCTTGCTCAGA.....
519	CATCACCCATCCCACCTCCAGCCCCA
468	.....
569	GC.....ACCACCAGCGCCC
469	GCATTGTTGTACAA.....
609	TGCCGACGCCGCAGCTCCTCCGTT
509	TG.CAAAAAACACAGACTC..GCGTT
657	AACCCAGACACCTGCAGGTGCCGGA
554	AACGAACGTACTTGCAGATGTGACA

Fig.4(iii)

20/52

Fig. 4(iv)

21/52

22/52	23/52	24/52
<i>Fig.5(i)</i>	<i>Fig.5(ii)</i>	<i>Fig.5(iii)</i>
25/52	26/52	27/52
<i>Fig.5(iv)</i>	<i>Fig.5(v)</i>	<i>Fig.5(vi)</i>

22/52

165SOMSQ.MSF.msf MSF:687  
 Type: D Tuesday, June 20, 1995  
 Check:3140

	1
VEGF165	ATGAACTTCTGCTGTCTGGGTG
SOM175	ATGAGCCCTCTGCTCCGCCGCCTG
SOM175-e6	ATGAGCCCTCTGCTCCGCCGCCTG
SOM175-e6&7	ATGAGCCCTCTGCTCCGCCGCCTG
SOM175-e4	ATGAGCCCTCTGCTCCGCCGCCTG
	81
VEGF165	CACCCATGGCAGAAGGAGGAGGGC
SOM175	TGCCCCTGGCCACCAGAGGAAAGT
SOM175-e6	TGCCCCTGGCCACCAGAGGAAAGT
SOM175-e6&7	TGCCCCTGGCCACCAGAGGAAAGT
SOM175-e4	TGCCCCTGGCCACCAGAGGAAAGT
	161
VEGF165	CCAATCGAGACCCTGGTGGACATC
SOM175	GTGGTGGTGCCCTTGACTG.TGGA
SOM175-e6	GTGGTGGTGCCCTTGACTG.TGGA
SOM175-e6&7	GTGGTGGTGCCCTTGACTG.TGGA
SOM175-e4	GTGGTGGTGCCCTTGACTG.TGGA
	241
VEGF165	GATGCGATGCCGGGGCTGCTGCAA
SOM175	GCAGCGCTGTGGTGGCTGCTGCC
SOM175-e6	GCAGCGCTGTGGTGGCTGCTGCC
SOM175-e6&7	GCAGCGCTGTGGTGGCTGCTGCC
SOM175-e4	GCAGCGCTGTGGTGGCTGCTGCC

*Fig.5(i)*

23/52

CATTGGAGCCTTGCCTGCTGCTCTACC  
CTGCTGCCCGCACTCCTGCAGCTGGCCC  
CTGCTGCCCGCACTCCTGCAGCTGGCCC  
CTGCTGCCCGCACTCCTGCAGCTGGCCC  
CTGCTGCCCGCACTCCTGCAGCTGGCCC

AGAACATCACGAAGTGGTGAAGTTCAT  
GGTGTCAATGGATAGATGTGTATACTCGC  
GGTGTCAATGGATAGATGTGTATACTCGC  
GGTGTCAATGGATAGATGTGTATACTCGC  
GGTGTCAATGGATAGATGTGTATACTCGC

TTCCAGGAGTACCCCTGATGAGATCGAGT  
GCTCATGGGCACCGTGGCCAAAC..AGC  
GCTCATGGGCACCGTGGCCAAAC..AGC  
GCTCATGGGCACCGTGGCCAAAC..AGC  
GCTCATGGGCACCGTGGCCAAAC..AGC

TGACGAGGCCTGGAGTGTGTGCCCACT  
TGACGATGGCCTGGAGTGTGTGCCCACT  
TGACGATGGCCTGGAGTGTGTGCCCACT  
TGACGATGGCCTGGAGTGTGTGCCCACT  
TGACGATGGCCTGGAGTGTGTGCCCACT

*Fig. 5(ii)*

24/52

80

TCCACCATGCCAAGTGGTCCCAGGCTG.  
CCGCCAGGCCCTGTCTCCCAGCCTGA  
CCGCCAGGCCCTGTCTCCCAGCCTGA  
CCGCCAGGCCCTGTCTCCCAGCCTGA  
CCGCCAGGCCCTGTCTCCCAGCCTGA

160

GGATGTCTATCAGCGCAGCTACTGCCAT  
G.....CTACCTGC.CAGCC.CCGGGAG  
G.....CTACCTGC.CAGCC.CCGGGAG  
G.....CTACCTGC.CAGCC.CCGGGAG  
G.....CTACCTGC.CAGCC.CCGGGAG

240

ACATCTTCAAGCCATCCTGTGTGCCCT  
TG GTGCCAG.....CTGCGTGACTGT  
TG GTGCCAG.....CTGCGTGACTGT  
TG GTGCCAG.....CTGCGTGACTGT  
TG GTGCCAG.....CTGCGTGACTGT

320

GAGGAGTCCAACATCACCATGCAGATTA  
GGGCAGCACCAAGTCCGGATGCAGATCC  
GGGCAGCACCAAGTCCGGATGCAGATCC  
GGGCAGCACCAAGTCCGGATGCAGATCC  
GGGCAGCACCAAGTCCGGATGCAGA...

*Fig.5(iii)*

25/52

321

VEGF165	TGC GGATCAAACCTCACCAAGGCC
SOM175	TCATGATCCGG...TACCCGAGCA
SOM175-e6	TCATGATCCGG...TACCCGAGCA
SOM175-e6&7	TCATGATCCGG...TACCCGAGCA
SOM175-e4	.....

401

VEGF165	AAGAAAGATA.G.....AGCAA
SOM175	AAAAAGGACAGTGCTGTGAAGCCA
SOM175-e6	AAAAAGGACAGTGCTGTGAAGCCA
SOM175-e6&7	AAAAAGGACAGTGCTGTGAAGCCA
SOM175-e4	AAAAAGGACAGTGCTGTGAAGCCA

481

VEGF165	.....AAGCA.....
SOM175	CTCTGCCCGGAGCACCCTCCCC
SOM175-e6	.....
SOM175-e6&7	.....
SOM175-e4	CTCTGCCCGGAGCACCCTCCCC

561

VEGF165	A.....GATCCGCA
SOM175	GCACCACCAGCGCCCTGACCCCCG
SOM175-E6	GCACCACCAGCGCCCTGACCCCCG
SOM175-e6&7	.....
SOM175-e4	GCACCACCAGCGCCCTGACCCCCG

641

VEGF165	TTGAGTTAACCGAACGTACTTGCA
SOM175	TAGAGCTCAACCCAGACACCTGCA
SOM175-e6	TAGAGCTCAACCCAGACACCTGCA
SOM175-e6&7	.....
SOM175-e4	TAGAGCTCAACCCAGACACCTGCA

Fig.5(iv)

26/52

AGCACATAGGAGAGATGAGCTTCCTACA  
GTCAGCTGGGGAGATGTCCCTGGAAGA  
GTCAGCTGGGGAGATGTCCCTGGAAGA  
GTCAGCTGGGGAGATGTCCCTGGAAGA  
.....

GACAAGAA . . . AATCCCTGTGG . . .  
GACAGGGCTGCCACTCCCCACCACCGTC  
GATAG . . . . . . . . . . . .  
GATAG . . . . . . . . . . . .  
GACAGGGCTGCCACTCCCCACCACCGTC

.....  
AGCTGACATCACCCATCCCACTCCAGCC  
..... CC  
.....  
AGCTGACATCACCCATCCCACTCCAGCC

GACGTGTAAATGTTCTGCACAAAC.AC  
GACCTGCCGCTGCCGCTGCCGACGCCGC  
GACCTGCCGCTGCCGCTGCCGACGCCGC  
.....  
GACCTGCCGCTGCCGCTGCCGACGCCGC

687

GATGTGACAAGCCGAGGCGGTGA  
GGTGCCGGAAGCTGCGAAGGTGA  
GGTGCCGGAAGCTGCGAAGGTGA  
. GTGCCGGAAGCTGCGAAGGTGA  
GGTGCCGGAAGCTGCGAAGGTGA

*Fig.5(v)*

27/52

400

GCACAAACAAATGTGAATGCAGACC...A  
ACACAGCCAGTGTGAATGCAGACCTAAA  
ACACAGCCAGTGTGAATGCAGACCTAAA  
ACACAGCCAGTGTGAATGCAGACCTAAA  
.....CCTAAA

480

.....GCCTTGCTCAGAGCGGAGA  
CCCAGCCCCGTTCTGTTCCGGGCTGGGA  
.....  
CCCAGCCCCGTTCTGTTCCGGGCTGGGA

560

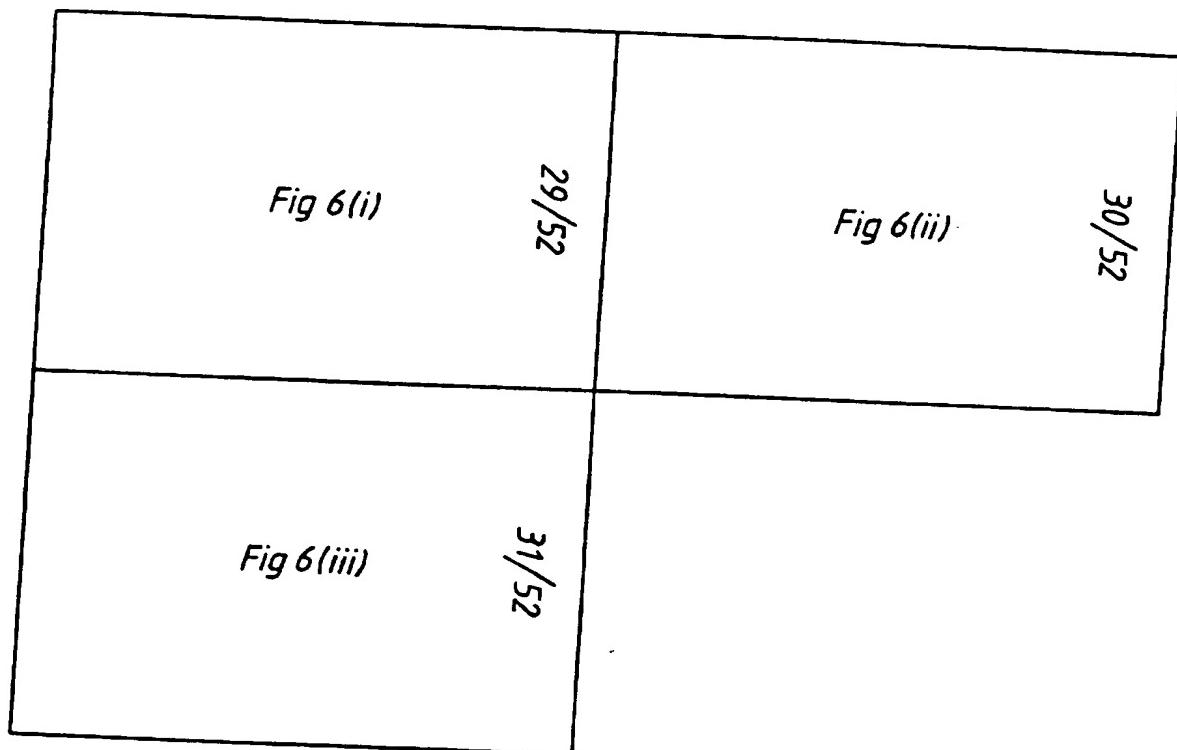
.....TTTGT.....TGTAC..A  
CCAGGGCCCTCTGCCAACGCTGCACCCA  
CCAGGGCCCTCTGCCAACGCTGCACCCA  
.....  
CCAGGGCCCTCTGCCAACGCTGCACCCA

640

AGACTCG..CGTTGCAAGGGCGAGGCAGC  
AGCTTCCTCCGTTGCCAAGGGCGGGGCT  
AGCTTCCTCCGTTGCCAAGGGCGGGGCT  
.....  
AGCTTCCTCCGTTGCCAAGGGCGGGGCT

*Fig.5(vi)*

28/52



30/52

29/52

VEGF <sub>165</sub>	M N F <b>L L</b> S W V H W S <b>L A L L Y L H H A K W S Q A A P</b>
SOM175 Short	M S P <b>L L</b> R R L L . . L A A L L Q <b>L A P A Q . . . A P</b>
VEGF <sub>165</sub>	I F Q <b>E Y P D E I E Y I F K P S C V P L M R C G G C C N</b>
SOM175 short	L T V <b>E L M G T V A K Q L V P S C V T V Q R C G G C C P</b>
VEGF <sub>165</sub>	F L Q <b>H N K C E C R P K K . . . . D R A . . . .</b>
SOM175 short	L E E H S Q <b>C E C R P K K . . . . D R A . . . .</b>
VEGF <sub>165</sub>	C K C S C K N T D S R C K A R Q L E L N E R T C R C D R H
SOM175 Short	H A A P S T T S A L T P G P A A A A D A A S S V A K
OR...	

VEGF <sub>165</sub>	M N F <b>L L</b> S W V H W S <b>L A L L Y L H H A K W S Q A A P</b>
SOM175 Long	M S P <b>L L</b> R R L L . . L A A L L Q <b>L A P A Q . . . A P</b>
VEGF <sub>165</sub>	I F Q <b>E Y P D E I E Y I F K P S C V P L M R C G G C C N</b>
SOM175 Long	L T V <b>E L M G T V A K Q L V P S C V T V Q R C G G C C P</b>
VEGF <sub>165</sub>	F L Q <b>H N K C E C R P K K . . . . D R A . . . .</b>
SOM175 Long	L E E H S Q <b>C E C R P K K . . . . D R A . . . .</b>
VEGF <sub>165</sub>	G P <b>C S E R R K H L F V Q D P Q T C K C S C K N T D S .</b>
SOM175 Long	P R C T Q H H Q R . . P D P R T C R C R C R R S F L

Fig.6(i)

M A E G G Q N	H H E .	V V K F M	D V Y Q R	S Y C H	P I E T L V	D 60
V S Q P D A P G	H Q R K V	V V S W I	D V Y T R A	T C C P R E	V V V V P	55
D E G I E C V P T	E E S N I	T M Q	I M R I K P H	Q G Q H I	G E M S	121
D D G I E C V P T	G Q H Q V	R M Q	I L M I R .	Y P S S Q L	G E M S	115
· · · · ·	· · · · ·	R Q E N P C G	C S E R R K	L F . V Q D P Q	T 170	
R P Q P R S V P G	W D S A P G A P	S P A D I T H	P T P A P G P	S A 175		
P R R						
G G A						

M	A	E	G	G	Q	N	H	H	E	.	V	V	K	F	M	D	V	Y	Q	R	S	Y	C	H	P	I	E	T	L	V	D	60			
V	S	Q	P	D	A	P	G	H	Q	R	K	V	V	V	V	D	V	Y	T	R	A	T	C	Q	P	R	E	V	V	V	P	55			
D	D	E	G	L	E	C	V	P	T	E	E	S	N	I	T	M	Q	I	M	R	I	K	P	H	Q	G	Q	H	I	G	E	M	S	121	
D	D	E	G	L	E	C	V	P	T	G	Q	H	Q	V	R	M	Q	I	L	M	I	R	.	Y	P	S	S	Q	L	G	E	M	S	115	
R	R	Q	E	N	P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	170				
R	R	P	Q	P	R	S	V	P	G	W	D	S	A	P	G	A	P	S	S	P	A	D	I	T	H	P	T	P	A	P	G	P	L	C	177
R	R	C	K	A	R	Q	L	E	L	N	E	R	T	C	R	C	D	K	P	R	R	L	E	L	N	P	D	T	C	R	K	L	R	R	191

THE SWEET BULLS

Fig. 6 (iii)

31/52

Areas of 100% homology are boxed and conserved residues thought to be involved in homodimerisation are underlined. The VEGF sequence depicted includes the 26 amino acid leader sequence (removal of which gives rise to mature VEGF<sub>165</sub>) giving a total length of 191 amino acids.

Homology of SOM175 to VEGF<sub>165</sub> is 27% (33%) at the protein level, however within this are blocks of 100% homology. In particular, many structural residues are conserved including those thought to be involved in homodimerisation of VEGF (by comparison with PDGF). i.e. Cysteine-47

Proline-70, Cysteine-72, Valine-74  
Arginine-77, Cysteine-78, Glycine-80, Cysteines-81 & 82  
Cysteines 122 & 124

Fig.6(iii)

32/52

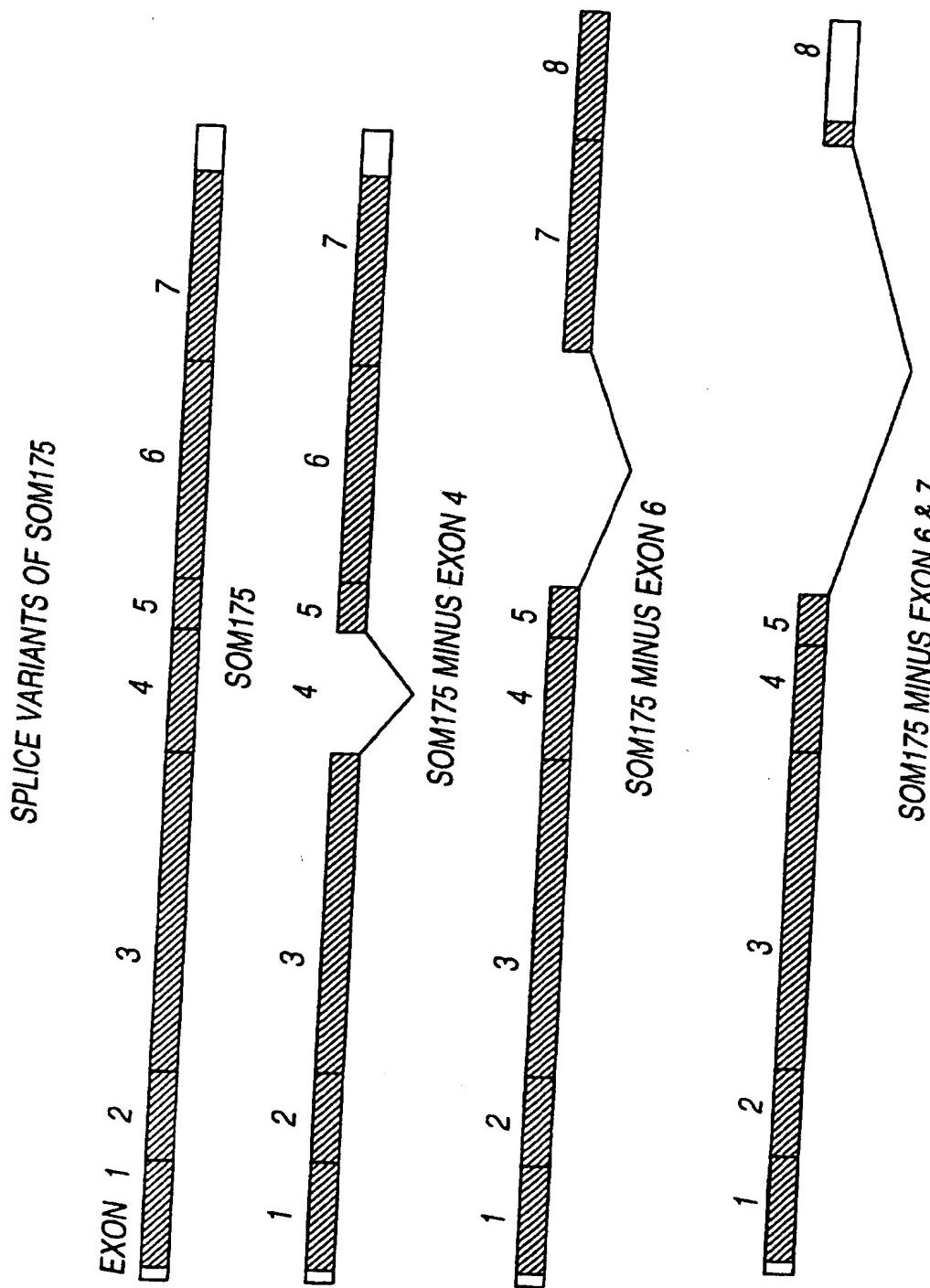


Fig. 7

33/52

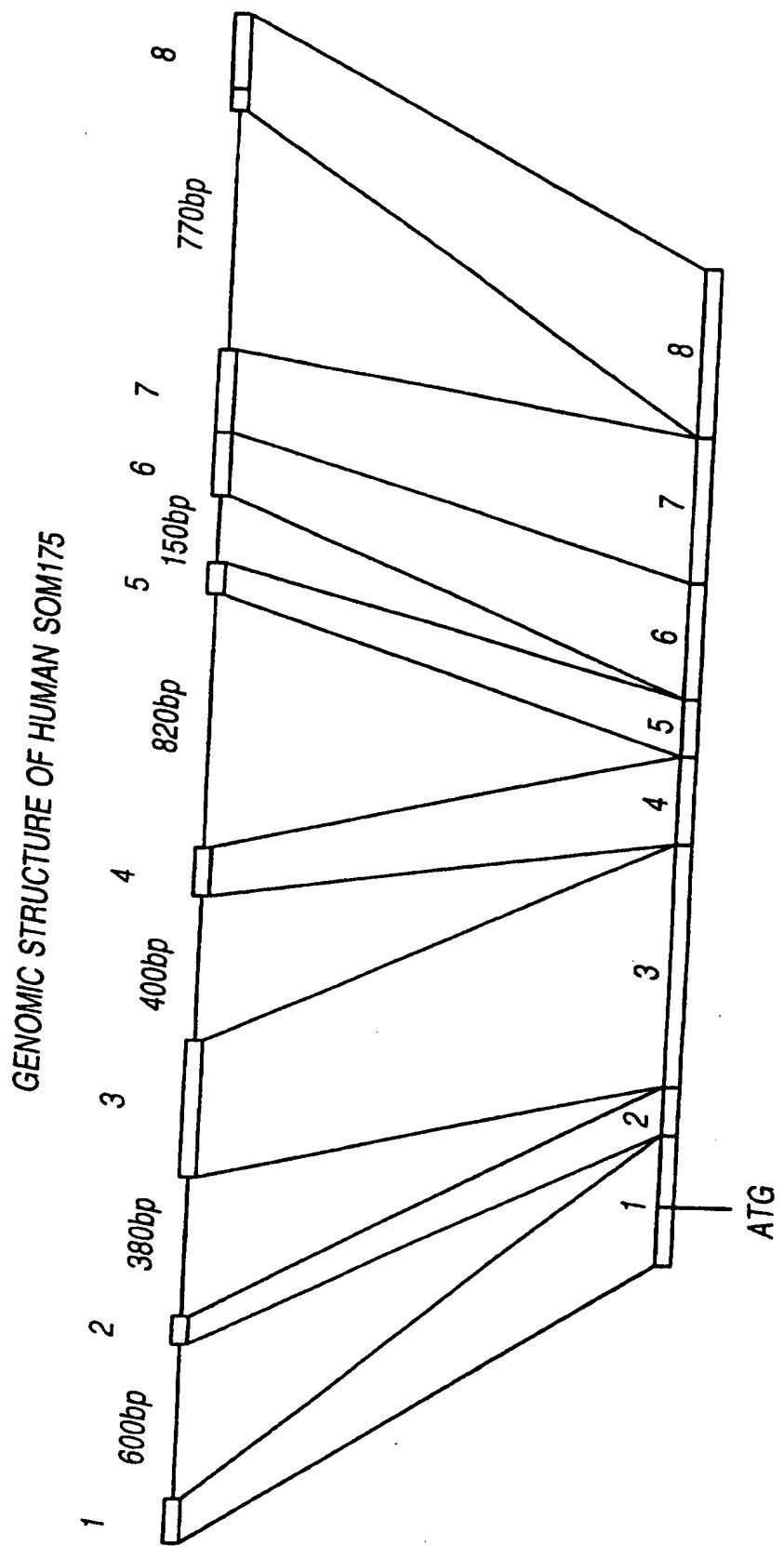


Fig. 8A

34/52

5' UTR . . .	<b>ATGAGG</b>	*Exon 1	(60bp)	GGCCAG gtacgtgagg
tctcccacag	GCCCCCT	Exon 2	(43bp)	GGAAAG aataacttaca
tctgctccca	TGGTGT	Exon 3	(187bp)	ATGCAG gtcccgagatg
ctgaatacag	ATCCTC	Exon 4	(73bp)	ATGCAG gtgtcaggca
actttcaag	ACCTAA	Exon 5	(34bp)	AGACAG gtgaggttt
ctcctccgta	GGCTGC	Exon 6	(101bp)	CTCCAG ccccaggccc
cccactccag	CCCCAG	Exon 7	(109bp)	ACCCAG acaccttag
ccctgctcag	GTGCCG	*Exon 8	(22bp)	<b>AGGTGA</b> . . . 3' UTR

*Fig. 8B*

35/52

36/52	37/52
<i>Fig. 9(i)</i>	<i>Fig. 9(ii)</i>
38/52	39/52
<i>Fig. 9(iii)</i>	<i>Fig. 9(iv)</i>

36/52

-163 gcacgagctcaggccgtcgctgcggcgctg  
 -103 gggggccgcggaggagccgcggccctgcgcc  
 -43 ggccggctctggctgacccccccacacccg

16 CGTCGCCTGCTGCTTGTGCAC TGCTGCAG  
     R R L L L V A L L Q

76 TTTGATGGCCCCAGTCACCAGAAGAAAGTG  
     F D G P S H Q K K V

136 ACATGCCAGCCCAGGGAGGTGGTGGTGCCT  
     T C Q P R E V V V P

196 AAACAAACTAGTCCCCAGCTGTGTGACTGTG  
     K Q L V P S C V T V

256 GGCCTGGAATGTGTGCCACTGGGCAACAC  
     G L E C V P T G Q H

316 TACCCGAGCAGTCAGCTGGGGAGATGTCC  
     Y P S S Q L G E M S

376 CCTAAAAAAAAGGAGAGTGCTGTGAGGCCA  
     P K K E S A V R P

436 CAGCCCCGCTCTGTTCCGGGCTGGGACTCT  
     Q P R S V P G W D S

*Fig.9(i)*

37/52

cgttgcgctgcctgcgcccagggctcgaaaa  
 ccgccccgggtccccgggtccgcgcacatgg  
 ccgggctagggccccATGAGCCCCCTGCTG  
 M S P L L -17  
 ↓  
 CTGGCTCGCACCCAGGCCCTGTGTCCCAG  
 L A R T Q A P V S Q 4  
 GTGCCATGGATAGACGTTATGCACGTGCC  
 V P W I D V Y A R A 24  
 CTGAGCATGGAACTCATGGCAATGTGGTC  
 L S M E L M G N V V 44  
 CAGCGCTGTGGTGGCTGCTGCCCTGACGAT  
 Q R C G G C C P D D 64  
 ↓  
 CAAGTCCGAATGCAGATCCTCATGATCCAG  
 Q V R M Q I L M I Q 84  
 ↓  
 CTGGGAGAACACAGCCAATGTGAATGCAGA  
 L G E H S Q C E C R 104  
 ↓  
 GACAGGGTTGCCATACCCCCACCACCGTCCC  
 D R V A I P H H R P 124  
 ACCCCCGGGAGCACCCCTCCCCAGCTGACATC  
 T P G A P S P A D I 144

*Fig.9(ii)*

*38/52*

---

496	<u>ATCCATCCCAC</u> TCCAGCCCCAGGATCCTCT
	I H P T P A P G S S
	S P R I L
556	CTGACCCCCGGACCTGCCGTTGCCGCTGTA
	L T P G P A V A A V
	P D P R T C R C R C
616	GGGGCT <u>TAGAGCTCAACCCAGACAC</u> CTGTA
	G A *
	R G L E L N P D T C
676	ctttccagactccacgggcccggctgcttt
736	agcacaggcgtaaacctcctcagtctggag
796	gagctctctcgccatcttttatctcccaga
856	atgtctcacctcagggccagggtactctc
916	ttctggctggctgtctcccctcaactatgaa
976	gggttctgttatgataactgtgacacacac
1036	gacactaaaaaaaaaaaaaaaaaaaaaaa

---

*Fig.9(iii)*

39/52

GCCCGCCTTGCACCCAGCGCCGCCAACGCC	
A R L A P S A A N A	164
C P P C T Q R R Q R	130

GACGCCGCCGCTTCCTCCATTGCCAAGGGC	
D A A A S S I A K G	184
R R R R F L H C Q G	150

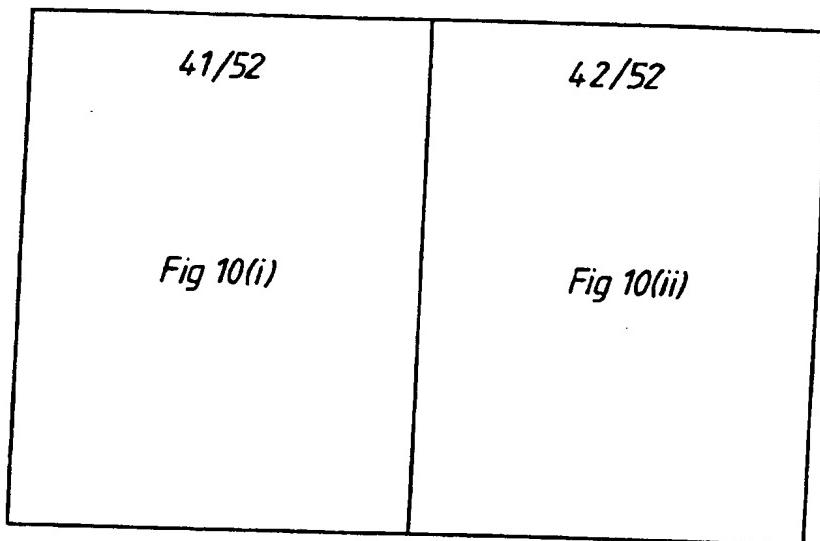
↓

GGTGCCGGAAGCCGCGAAAGT <u>G</u> Acaagctg	
R C R K P R K *	186
	167

tatggccctgcttcacagggagaagagtggtgg	
gtcactgccccaggacctggaccttttaga	
gctgccatctaacaattgtcaaggaacctc	
tcacttaaccaccctggtcaagtgagcatc	
aaccccaaacttctaccaataacgggattt	
<u>acacactcacactctgataaaaagagatgga</u>	
aaaaaaaaaaaaaa	

Fig.9(iv)

40/52



41/52

## A

hVRF167	-21	MSPLLRRLLL AALLQLAPAQAP	↑
mVRF167	-21	MSPLLRRLLL VALLQLARTQAP	
hVRF167	30	EVVVPLTVELMGTVAKQLVPSC	
mVRF167	30	EVVVPLSMELMGNVVKQLVPSC	
hVRF167	80	ILMIRYPSSQLGEMSLEEHSQLC	
mVRF167	80	ILMIQYPSSQLGEMSLGEHSQC	
hVRF167	130	RPDPRTCRCRCRRRSFLRCQGR	
mVRF167	130	RPDPRTCRCRCRRRFLLHCQGR	

## B

hVRF186	116	RAATPHHRPQPRSVPGWDSAPG	
mVRF186	116	RVAIPHHRPQPRSVPGDSTPG	
hVRF186	166	TPGPAAAAADAAAASSVAKGGA*	
mVRF186	166	TPGPAVAAVDAAASSIAKGGA*	

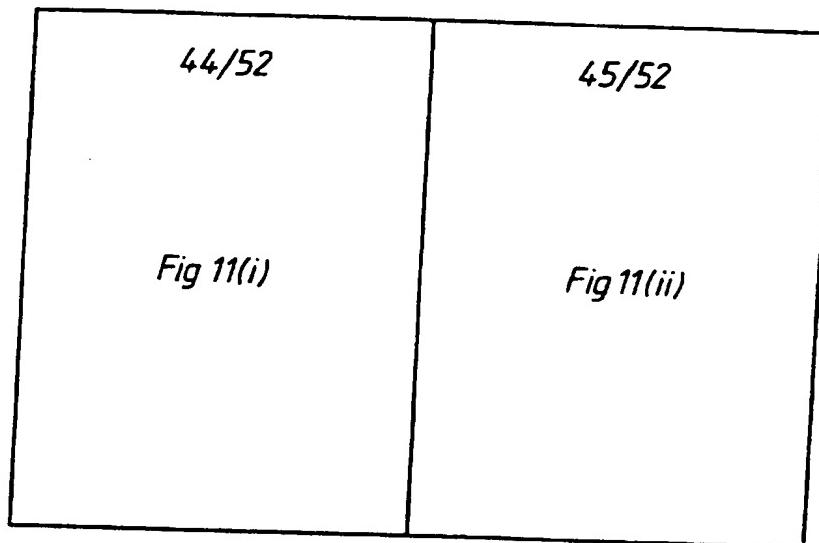
*Fig. 10(i)*

42/52

VSQPDAPGHQRKVVSWIDVYTRATCQPR	29
:    :	
VSQFDGPGSHQKKVVVPWIDVYARATCQPR	29
VTVQRCGGCCPDDGLECVPTGQHQVRMQ	79
VTVQRCGGCCPDDGLECVPTGQHQVRMQ	79
ECRPKKKDSAVKPDSRPLCPRCTQHHQ	129
:      :                   :	
ECRPKKKESAVRPDSRILCPPCTQRRQ	129
GLELNPDTCRCKLRR*	167
:	
GLELNPDTCRCKPRK*	167
PSPADITHPTPAPGPSAHAAPSTTSAL	165
:	
PSPADIIHPTPAPGSSARLAPSAAANAL	165

*Fig. 10(ii)*

43/52



44/52

mVRF167	-21	MSPLLRRRL..LLVALLQL..	
		::   :	
mVEGF188	-26	MNFLLSWVHWTLALLLYLHH	
mVRF167	25	TCQPREVVVPLSMELMGNVV	
		:   :   : :: :	
mVEGF188	24	YCRPIETLVDIFQEYPDEIE	
mVRF167	75	QVRMQILMIQYPSSQ.LGEM	
		:     :     :	
mVEGF188	74	NITMQIMRIKPHQSQHIGEM	
mVRF167	119	.....ILCPPC	
		:	
mVEGF188	124	QKRKRKKSRFKSWSVHCEPC	
mVRF167	152	GLELNPDTCRCKPRK	
		:	
mVEGF188	173	QLELNERTCRCDKPRR	

Fig.11(i)

45/52

*Fig. 11(ii)*

46/52

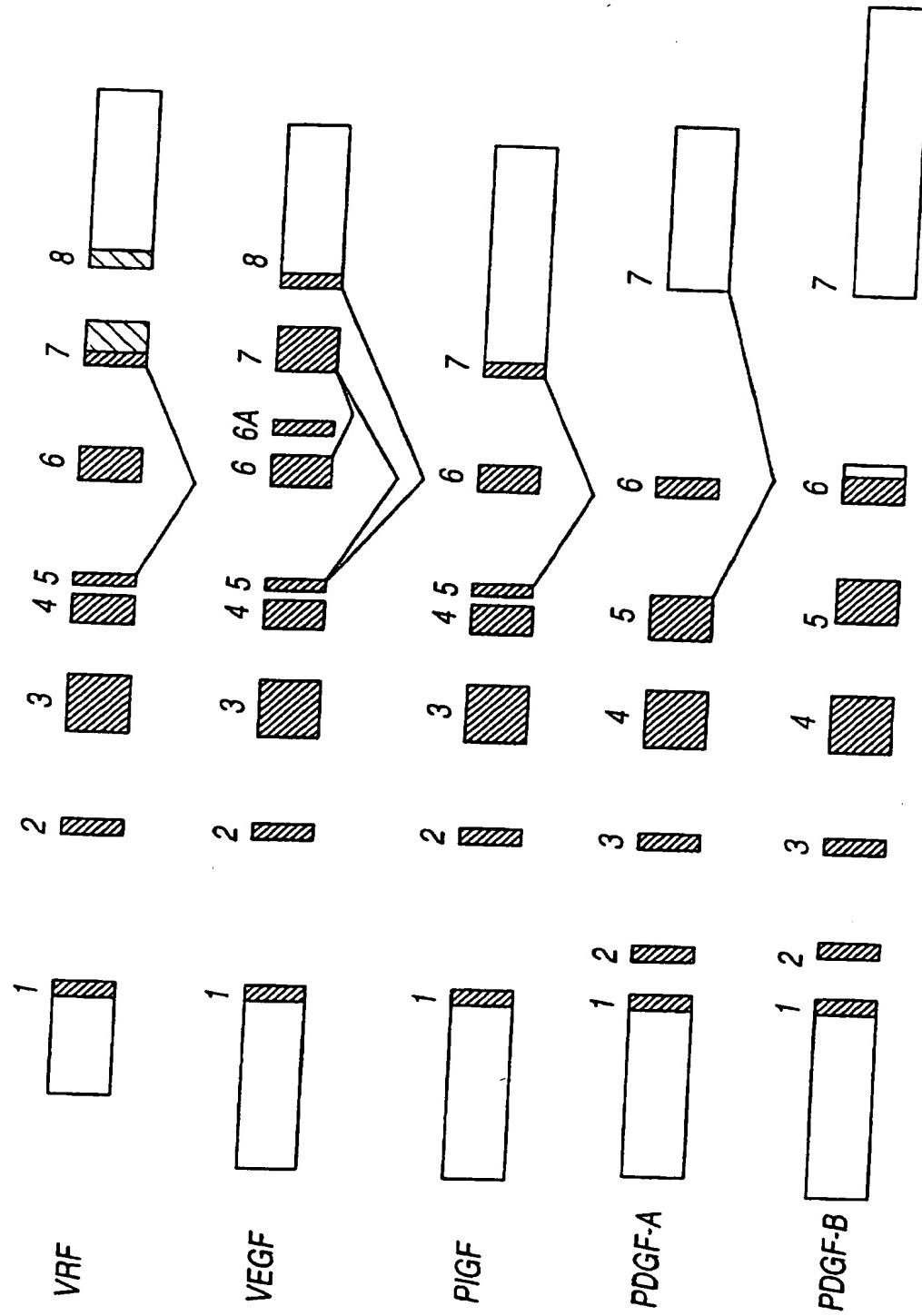
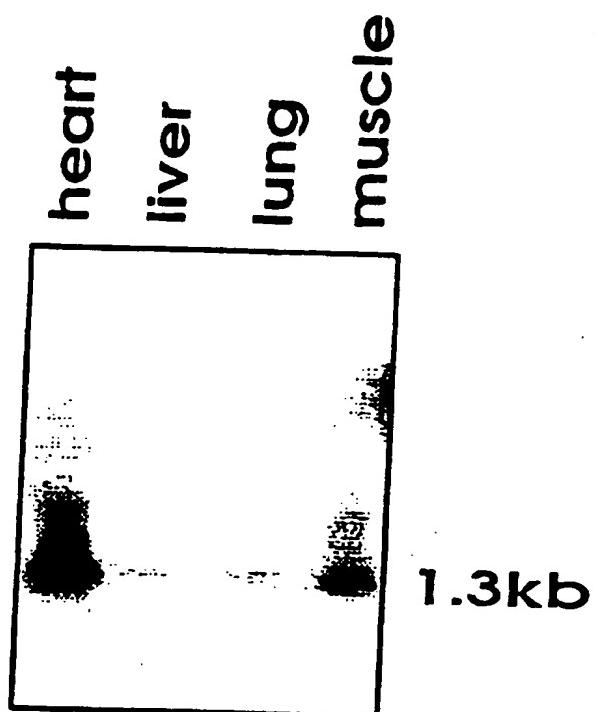


Fig.12

47/52



*Fig. 13*

48/52

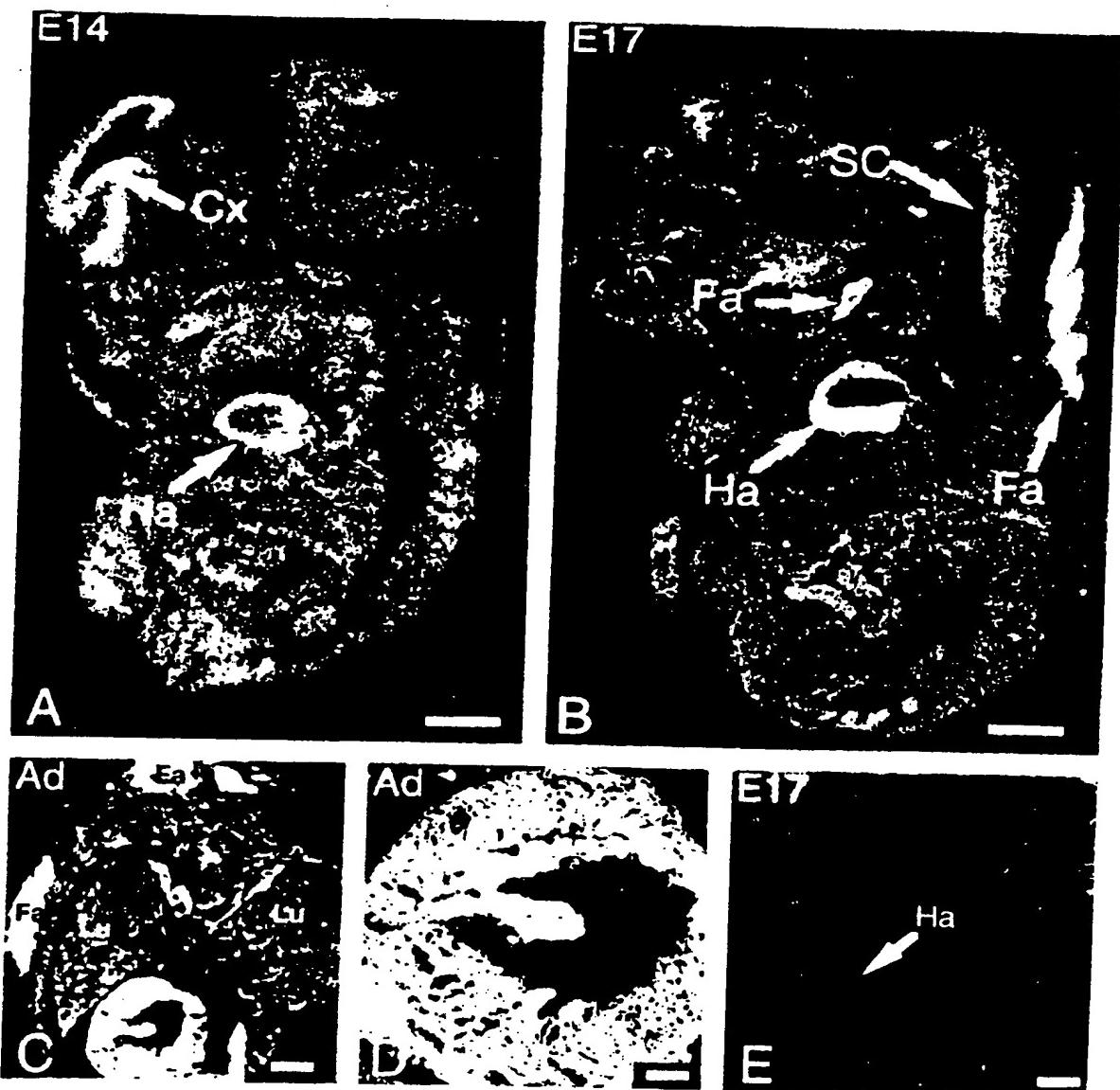


Fig.14

49/52

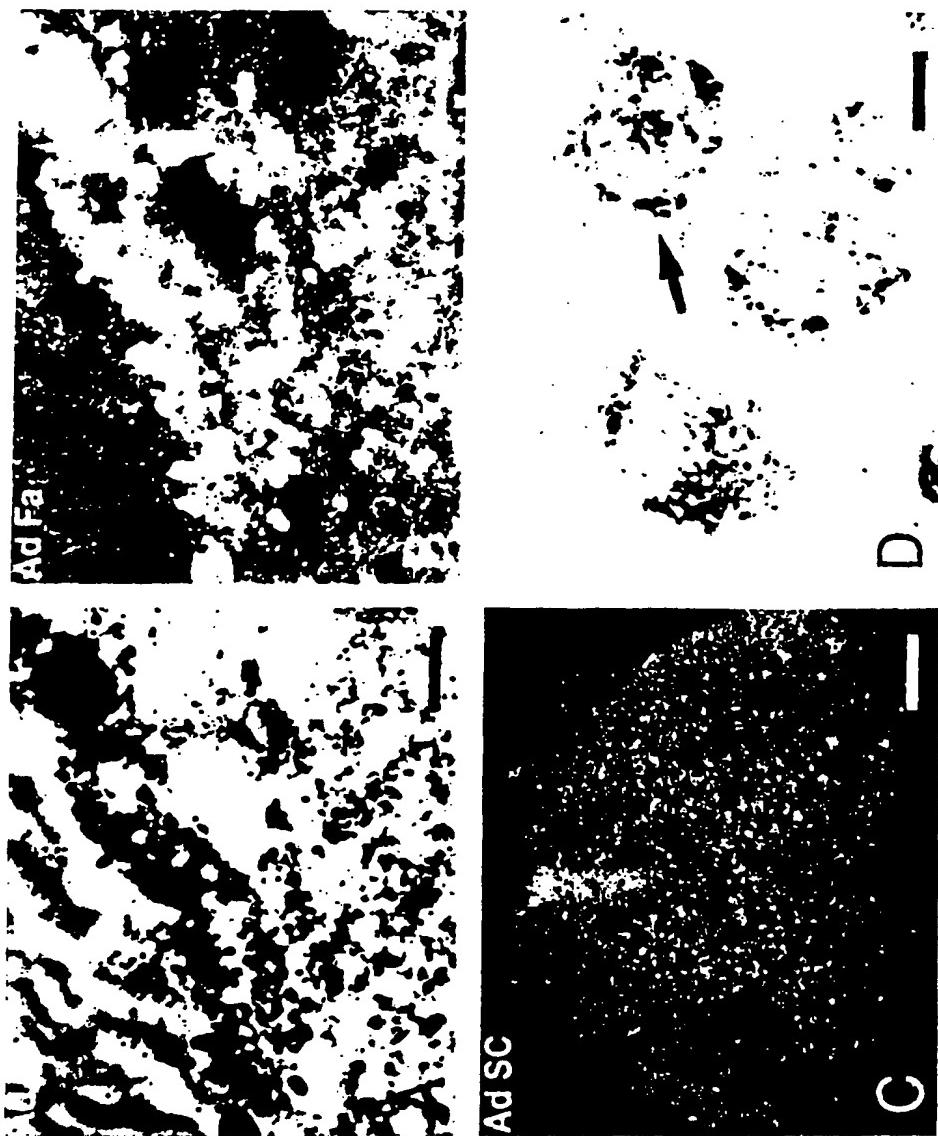


Fig. 15

50/52

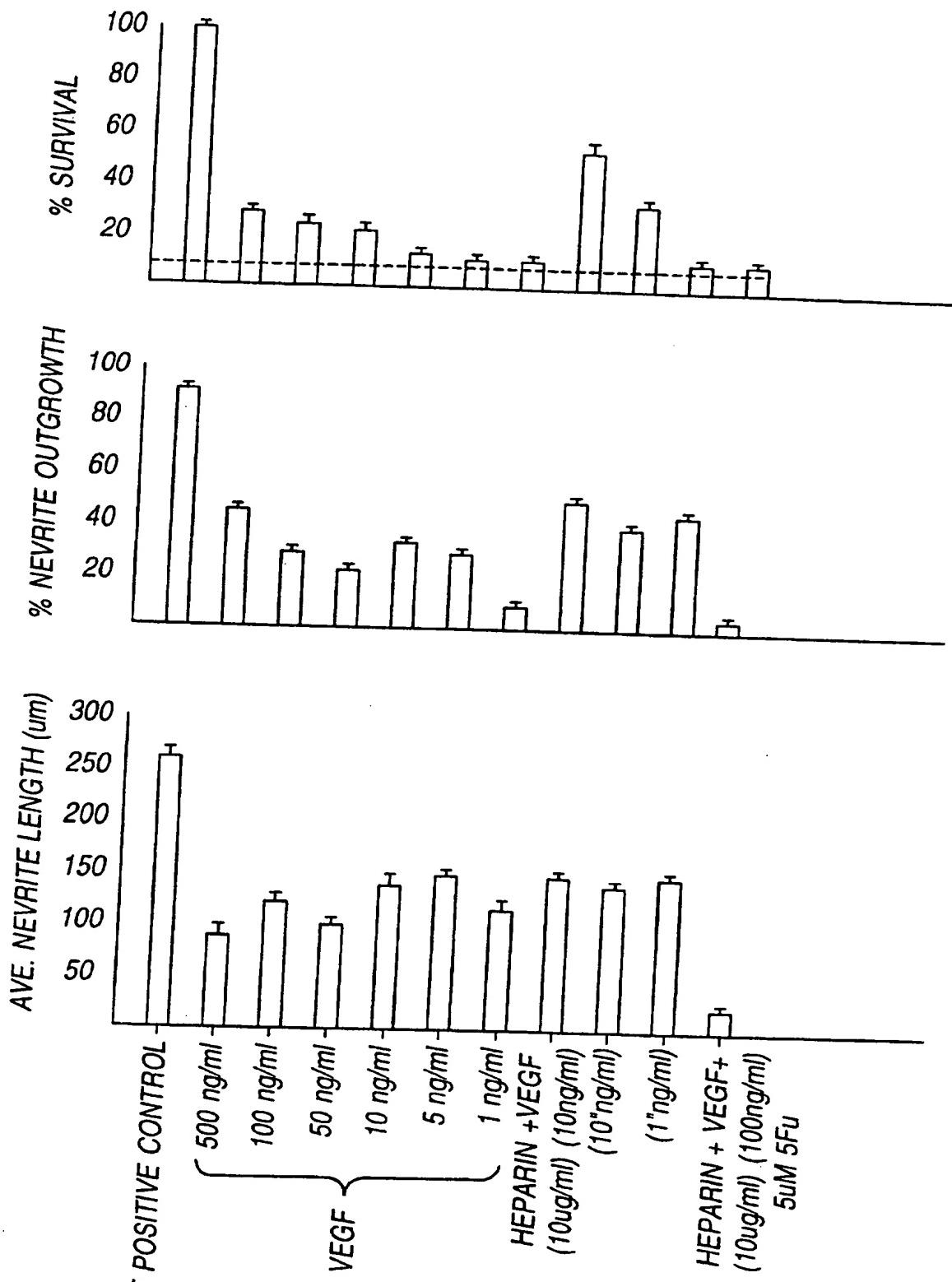


Fig. 16

51/52

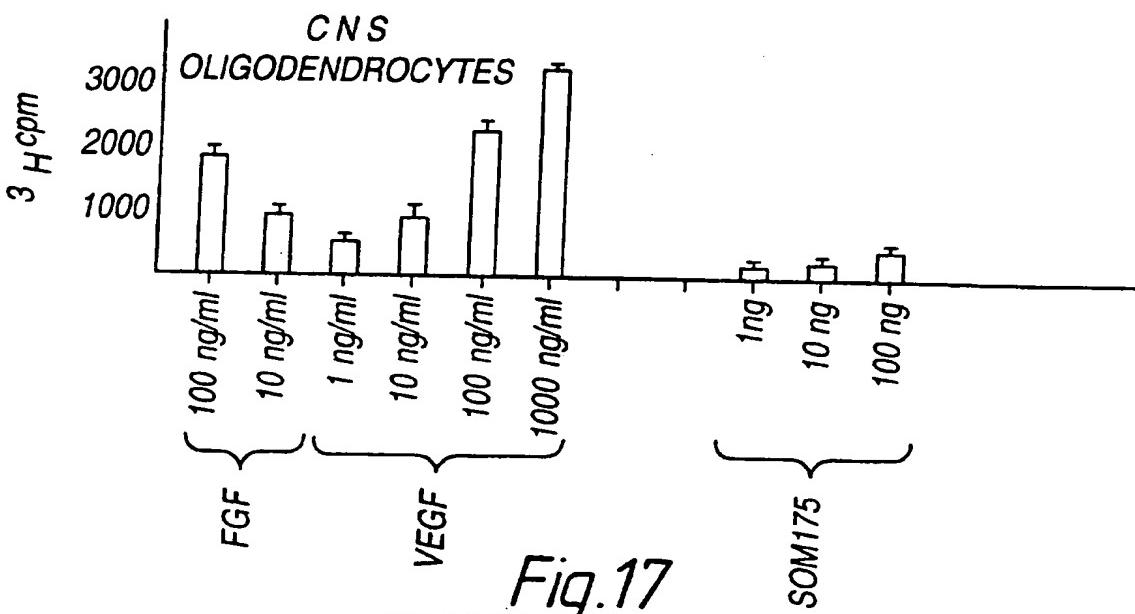
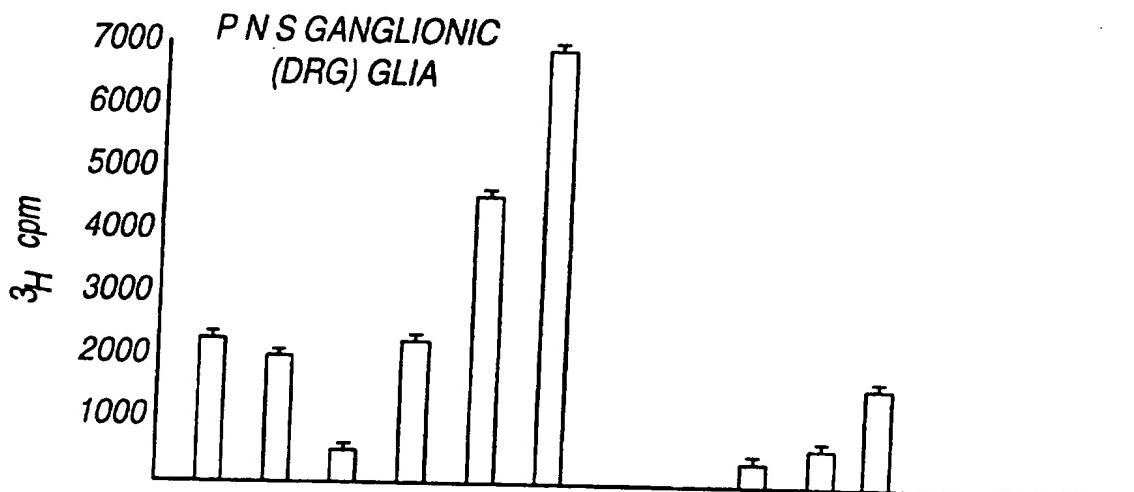
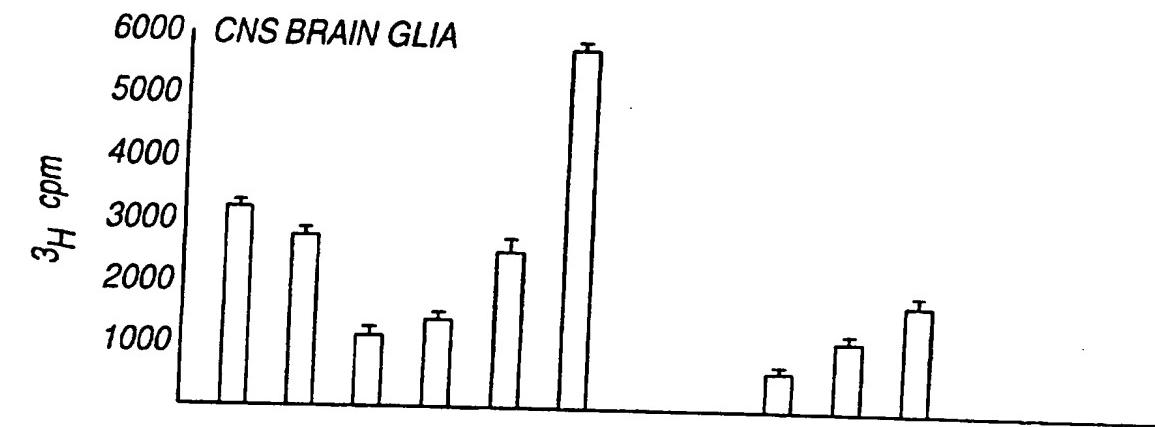
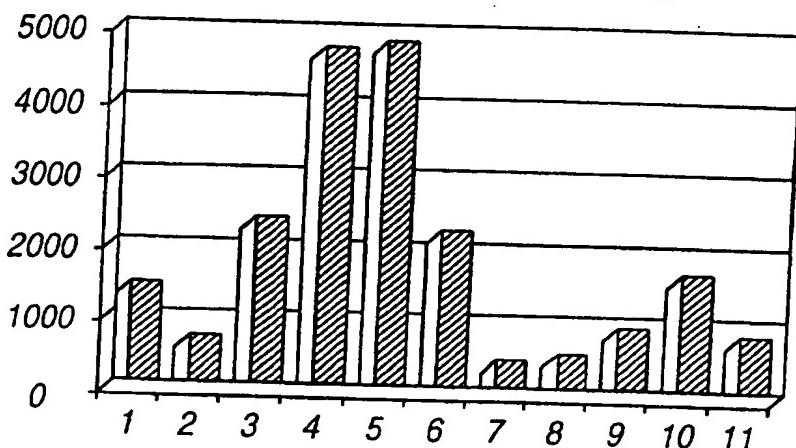
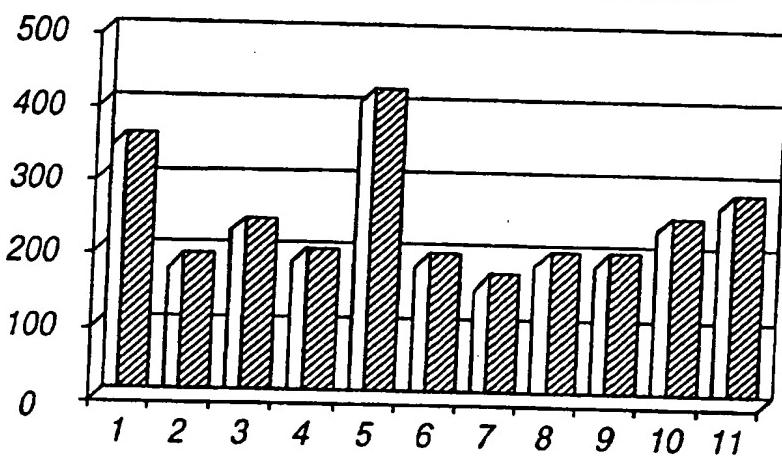


Fig.17  
SUBSTITUTE SHEET (RULE 26)

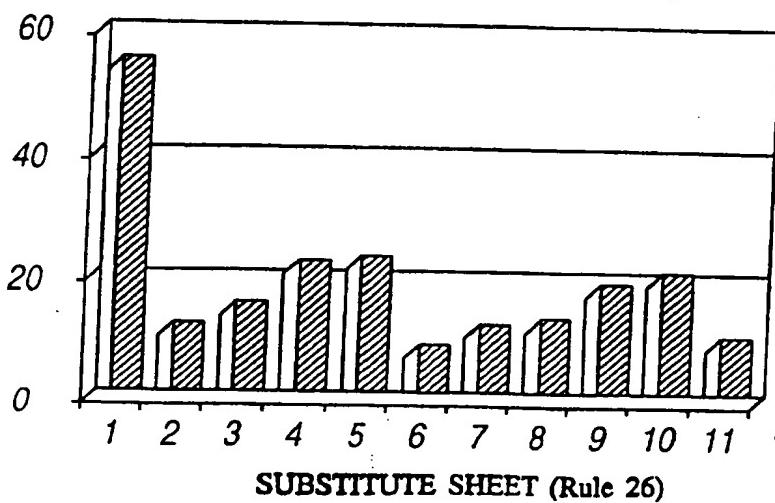
*52/52*  
MOUSE ASTROGLIAL CELLS

*Fig.18*

MOUSE OLIGODENDROGLIAL CELLS

*Fig.19*

MOUSE FOREBRAIN NEURONS

*Fig.20*

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/AU 96/00094

## A. CLASSIFICATION OF SUBJECT MATTER

Int Cl<sup>6</sup>: C12N 15/12; C07K 14/475; A61K 037/02

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
**WPAT AND CHEM ABS**

**SEE DETAILS IN ELECTRONIC DATABASE BOX BELOW**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
**USPM, JADIO**

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
**DERWENT WPAT, USPM, JADIO DATABASES; KEYWORDS: MVRF OR HVRF OR SOM 1: OR SOM X: OR [VASOACTIVE () PERMEABILITY () FACTOR#] OR VEGF: OR VEGF OR VRF: OR VRF OR [GROWTH () FACTOR (5N) VASCULAR OR ENDOTHELI:] CHEMICAL ABSTRACTS DATABASE; KEYWORDS: AS ABOVE**

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU 60798/90 (CALIFORNIA BIOTECHNOLOGY INC) published 21 February 1991	1-41
X	AU 56574/90 (GENENTECH, INC.) published 15 November 1990	1-41
P,X	AU 73941/94 (HUMAN GENOME SCIENCES, INC.) published 14 September 1995	1-41

Further documents are listed in the continuation of Box C

See patent family annex

• Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search  
03 June 1996

Date of mailing of the international search report

5<sup>th</sup> June 1996.

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INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/AU 96/00094

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5073492 (Chung-Ho Chen and Sumi C. Chen) published 17 December 1991	34
X	Biochemical and Biophysical Research Communications (1992), Vol 183, No. 3, pg 1167-1174 (Weindel K. et al.) "AIDS-ASSOCIATED KAPOSI'S SARCOMA CELLS IN CULTURE EXPRESS VASCULAR ENDOTHELIAL GROWTH FACTOR". See whole Article.	1-41
X	Biochemical and Biophysical Research Communications (1989), Vol 165, No. 3 pg 1198-1206 (Edmund Tischer et al.) "VASCULAR ENDOTHELIAL GROWTH FACTOR : A NEW MEMBER OF THE PLATELET-DERIVED GROWTH FACTOR GENE FAMILY". See whole Article.	1-41
X	Journal of Virology (1994), Vol 68, No. 1 pg 84-92 (David J. Lytle et al.) "HOMOLOGS OF VASCULAR ENDOTHELIAL GROWTH FACTOR ARE ENCODED BY THE POX VIRUS ORF VIRUS". See whole Article.	1-41
X	Methods in Enzymology (1991), vol 198, pg 391-405 (Ferrara Napoliana et al.) "PURIFICATION AND CLONING OF VASCULAR ENDOTHELIAL GROWTH FACTOR SECRETED BY PITUITARY FOLLICULOSTELLATE CELLS". See whole Article	1-41
X	The Journal of Biological Chemistry (1991), vol 266, No. 18 pg 11947-11954 (Edmund Tischer et al.) "THE HUMAN GENE FOR VASCULAR ENDOTHELIAL GROWTH FACTOR". See whole Article	1-41
P,X	Biochemical and Biophysical Research Communications (1996), Vol 220, No. 1 pg 147-52 (Lagercrantz J et al) "EXPRESSION OF THE VEGF-RELATED FACTOR GENE IN PRE-AND POSTNATAL MOUSE". See whole Article	33
P,X	Biochimica et Biophysica Acta (1995), Vol. 1260 No. 2 pg 235-9 (Sharma Hari S et al.) "NUCLEOTIDE SEQUENCE AND EXPRESSION OF THE PORCINE VASCULAR ENDOTHELIAL GROWTH FACTOR". See whole Article.	1-41
X	DEVELOPMENT (1992), Vol 114, pg 521-532 (Breier G et al.) "EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR DURING EMBRYONIC ANGIOGENESIS AND ENDOTHELIAL CELL DIFFERENTIATION". See whole Article	1-41
X	Molecular Endocrinology (1991), Vol 5 No. 12, pg 1806-1814 (Houck Keith A et al.) "THE VASCULAR ENDOTHELIAL GROWTH FACTOR FAMILY: IDENTIFICATION OF A FOURTH MOLECULAR SPECIES AND CHARACTERIZATION OF ALTERNATIVE SPLICING OF RNA". See whole Article	1-41

**INTERNATIONAL SEARCH REPORT**International Application No.  
PCT/AU 96/00094

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
AU	56574/90	US IL WO	5332671 94128 9013649	CA JP	2054699 4505705	EP NZ	471754 233451
AU	60798/90	US JP	5194596 5501350	CA WO	2063810 9102058	EP US	484401 5219739
AU	73941/94	WO	9524473				
US	5073492	NONE					

END OF ANNEX